1 In Environmental Microbiology, 2020

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3 **Multi-model assessment of hydrological and Environmental**
4 **impacts on streambed microbes in Mediterranean catchments**

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8 **SUMMARY**

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10 Microbes inhabiting intermittent streambeds are responsible for controlling and developing
11 many biogeochemical processes essential for the ecosystem functions. Although streambed
12 microbiota is adapted to intermittency the intensification of water scarcity and prolonged dry
13 periods may jeopardise their capacity to cope with hydrological changes. This study aims to
14 evaluate whether, and to what extent, the duration of dry periods affects streambed microbial
15 density, diversity, composition (16S rRNA gene diversity) and functions (extracellular
16 enzyme activities and respiration). Our results highlight the fact that hydrology modulates the
17 community composition and, to some extent, the functions carried out under different
18 environmental conditions. The relative abundance of certain taxa inhabiting the driest
19 intermittent communities differs significantly from those found at sites with continuous flow.
20 Microbial functional metrics revealed a progressive increase in recalcitrant carbon
21 degradation activity at sites with an extended dry phase. In contrast, bacterial density and
22 diversity were mainly influenced by the catchment land use, agriculture enhanced density but
23 reduced diversity, and the presence of riparian vegetation supported greater streambed
24 bacterial diversity. From this perspective, a combination of prolonged dryness with reduced
25 riparian vegetation and increased agricultural land cover could compromise the ecosystem
26 functioning by threaten microbially mediated processes linked to the carbon cycle.

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28 **INTRODUCTION**

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Dry streambed sediments are dynamic ecotones where terrestrial and aquatic ecosystems interact, their functioning is largely driven by microorganisms hosted in the sediment and shaped by temporal environmental fluctuations (Datry et al., 2017; Arce et al., 2019). Currently, dry streambeds are expanding worldwide due to increased flow intermittency. Reduced annual precipitation, increased water extraction and the global temperature rise are the major factors intensifying flow intermittency, especially in Mediterranean areas (Cipriani et al., 2014; Schewe et al., 2014). Most Mediterranean intermittent streams, such as those in the northeastern Iberian Peninsula, usually cease flowing for between 1 and 2 months each year in the summer period. It has been reported that, currently, intermittent streams may exhibit extended dry-phase episodes also coupled with intense and sporadic precipitation (Duran et al., 2017). The increase of streambed aridity in intermittent streams and the consecutive reduction of sediment-water content due to extended exposure to the air may affect the hosted microbiota and compromise microbial metabolism (Rees et al., 2006; Marxsen et al., 2010; Febria et al., 2015; Gionchetta et al., 2019). Microbial streambed communities are responsible for key ecosystem processes such as nutrient cycling and biogeochemical processes, and their metabolic capabilities are fundamental for maintaining fluvial ecosystem functioning (Datry et al., 2017). Studies report that a long period of water stress reduces specific microbial activities linked to carbon degradation, probably due to: (i) the transition of microbes to a dormant stage (Schimel et al., 2007; Jones and Lennon, 2010); (ii) the allocation of a large fraction of cell metabolic costs for developing survival strategies to cope with desiccation (such as the production of extracellular polymeric substances) (Lake, 2003; Frossard et al., 2012; Romaní et al., 2017); and/or (iii) reduced labile organic matter available for microbes during droughts and increased accumulation of recalcitrant compounds on the streambed surface (Ylla et al., 2010; Freixa et al., 2016). On the other hand, and in a similar way to arid soil systems, frequent or sporadic precipitations interrupting dry periods can cause sharp exceptional increases in streambed sediment respiration activity, as a result of the awakening of dormant microbes (Bérard et al., 2011; Evans et al., 2014; Meisner et al., 2017; Marcé et al., 2019). Streambed microbial assemblages have been shown to be affected by drying–rewetting cycles, especially when these include unusually extended dry phases (Pohlon et al., 2013; Meisner et al., 2018). Their taxonomic composition can shift towards a community able to cope with more extreme environmental conditions, resulting in a more

specialized community able to develop specific life strategies for coping with drought (Jones and Lennon, 2010; Shade et al., 2012; Evans and Wallenstein, 2014). In addition to hydrology, streambed microbes in intermittent systems may be further affected by co-occurring environmental factors related to the characteristics of the catchment acting at different spatial scales. Physical characteristics, such as the type of streambed substrata (e.g. cobbles/bedrock, fine/coarse sediment, accumulation of leaf litter), can influence the microbial response to hydrological intermittency (Duarte et al., 2017; Arce et al., 2019). Headwaters are usually more heavily affected by natural intermittency than downstream areas, due to their hydro-morphological features (e.g. pronounced slope, distinct geomorphology reducing the hyporheic zone); also, these areas have been described as microbial biodiversity reservoirs, reflecting the high variability of habitats and resources (varying quantity and quality of organic matter), and/or higher levels of microbial colonization from the adjacent soils than that observed downstream (Besemer et al., 2013). In headwater sections, a greater accumulation of leaf litter combined with a reduced percentage of sand and gravel sediment particles could lessen the loss of organic matter and water content during dry periods, limiting the reduction of microbial activity (Freixa et al., 2016). Other studies have reported that leaf packs buried in the sediment maintain high humidity under extreme dry conditions, becoming temporal shelters for microbial survival (Gionchetta et al., 2019). Similarly, the presence of riparian vegetation, able to curtail water loss from sediments under dry conditions, has been considered a hydrological refuge for microbiota inhabiting the streambed (McLaughlin et al., 2017). At a larger scale, catchment land uses can also influence the stream and streambed biota and microbiota responses, especially through factors relating to the use of the surrounding soils (e.g. agriculture or industrial activities), or a reduction in the natural riparian vegetation (Allan, 2004; Steward et al., 2012, 2018; Bruno et al., 2016). To date, most studies related to the consequences of intermittency on streambed microbes have been mainly focused on the effects provoked by wet (and rewetting) periods (Timoner et al., 2014). Less attention has been paid to the drought period, with only a few field studies focused on a single or limited number of stream ecosystems (Timoner et al., 2012; Zeglin, 2015; Freixa et al., 2016). However, to better understand streambed microbial responses to intermittency, field studies embracing the multiple pressures (including the hydrological history and other environmental factors) are needed, as shown by recent studies demonstrating the interactive and joint effect

of multiple factors on biological communities in natural ecosystems (Besemer et al., 2013; Dickie et al., 2015; Bruno et al., 2016; Gieswein et al., 2017). Accordingly, this work has attempted to fill this gap by evaluating the functional and structural responses of streambed microbial communities to a natural dryness gradient, by studying intermittent streams located in the Mediterranean basin. As the duration of dry and wet periods is highly unpredictable, the hydrology of each of the 37 selected sites was monitored in situ and later classified based on the natural gradient of their dryness history. From the 8 months of hydrology recorded, several parameters were calculated, including the duration of the total dry phase, as well as the length of the last wet event before our sampling. In parallel, a range of environmental factors were measured and included as potential predictors for the responses of the streambed microbial community (e.g. riparian vegetation, organic matter content and land use). Specifically, this investigation aimed to (i) elucidate whether, and to what extent, the hydrological history has influenced the microbial diversity, composition and activity of sediment microbial communities in the selected Mediterranean intermittent streams; (ii) determine the relative contribution of catchment and sediment characteristics (riparian vegetation, organic matter and water content, and land use) to modulating the microbial diversity, composition and activity according to the various hydrological factors; and (iii) define patterns of, and linkages between, the composition of the bacterial community and its metabolic activities along the dryness gradient. At sites with long dry-phases, we expected decreased microbial labile carbon degradation capacities but increased recalcitrant material degradation capacities, together with a transition of the microbial community composition towards a soil-like assemblage. We hypothesized to observe clearer microbial functional responses to the changes of sediment-water content compared with those related to the microbial composition and density.

RESULTS

Site classification and catchment characteristics

The principal component analysis (PCA) clearly distinguished the hydrological and catchment characteristics along the first and second axes respectively (Fig. 1). Factors with correlation

coefficients >0.80 (Pearson correlation, r_p) with the first two PCA components (PC1 and PC2) were selected to define gradients between sites. The first axis accounted for 40% of the total variability, describing the increase in both the total dry duration (T_DRY, r_p : 0.941, Fig. 1) and the percentage of dry phase duration (P_DRY, r_p : 0.941, Fig. 1), as well as the concurrent decrease (in the opposite PC1 direction) in the number of consecutive wet days prior to the sampling (REW, r_p : -0.946 , Fig. 1). The variability along the second axis, explaining 17.3% of the total variation, mainly corresponded to the increase in forested land cover (FORE, r_p : 0.861, Fig. 1) and the decrease (in the opposite PC2 direction of the) in agricultural land-use (AGRI, r_p : -0.888 , Fig. 1). The site distribution was clearly ordinated along PC1, which identified four hydrological history categories (FL, flow sites; SD, short-dry phase sites; MD, medium-dry phase sites; LD, long-dry phase sites see Experimental procedures section) based on the duration of their dry phase. The PC2 axis ordered the sites according to their forested or agricultural land cover (Table 1). Hydrology and catchment characteristics were the main drivers shaping the site distribution, where LD-phase sites had significantly lower riparian vegetation (RIP_VEG) and higher organic matter content (ORG_MAT) compared with FL sites ($p < 0.05$, Table 1). All the sites showed similar percentages of sediment water content since the sampling was conducted over the first days of the wet season (WAT_CON, Table 1). As expected, LD sites showed the longest dry period (L_DRY) prior to the sampling and the shortest rewetting periods (REW) in comparison to the other sites ($p < 0.05$, Table 1). A similar frequency of dry/rewetting cycles was observed between the sites, and only MD sites presented significantly higher frequencies than those of FL sites ($p < 0.05$, Table 1).

Influence of hydrological history on streambed microbial functions

The streambed extracellular enzymes and community respiration were not significantly different between the four hydrological categories (Fig. S2), but their variability was particularly high for SD sites, in the case of hydrolytic activities (β -glucosidase, GLU and β -xylosidase, XYL), and for MD and LD sites, in the case of sediment respiration (Fig. S2). Both variance partitioning (Fig. S3) and averaged models (Table 2) indicated that hydrological variables were important for phenoloxidase (PHE) and community respiration (RESP), while catchment characteristics explained more of GLU and XYL variability (Fig.

S3, Table 2). Specifically, the total duration of the dry phase explained the largest variance in the case of PHE activity (PHE, VAR. PAR. T_DRY = 13%, Fig. S3), while the interaction between the total dry duration (T_DRY) and the length of the last wet period (REW) influenced the variation in community respiration (RESP, VAR. PAR. T_DRY*REW = 13%, Fig. S3). In contrast, the organic matter content and riparian vegetation cover better explained the variance of XYL (VAR. PAR. ORG_MAT = 22%, RIP_VEG = 25%) while both organic matter and water content of sediments were relevant for GLU variance explanation (VAR. PAR. ORG_MAT = 17%, WAT_CON = 15%, Fig. S3). Microbial function plots showed the relationship (significant or not) between each functional variable and the total dry duration as well as the effect (e.g. additive, synergistic or antagonist) of the duration of the last wet period (Fig. 2). According to the model plots, despite the high variability seen between the intermittent sites, the hydrolytic GLU and XYL activities decreased slightly when T_DRY increased, and this trend was significant in the case of XYL activity (Fig. 2A and B, Table 2). In contrast, PHE activity increased significantly with longer dry periods, with mean PHE activities doubling at LD sites compared with those at SD sites (126.63 $\pm$ 13.64 and 251.13 $\pm$ 55.19 nmol DIQC gDW⁻¹ h, at SD and LD sites respectively), while no interaction with REW was found (Fig. 2C, Fig. S2 and Table 2). With regard to catchment characteristics, sediment organic matter content was positively related to GLU and XYL hydrolytic activities (Table 2), while the riparian vegetation cover was negatively related to XYL activity (Table 2). Community respiration presented a more complex response to the hydrological conditions, with peaks occurring with longer rewetting periods in largely intermittent and extremely dry sediments (MD and LD sites in Fig. 2D, Table 2).

Streambed microbial diversity responses to hydrological history and relationship to function Remarkably, the sediment microbial community diversity metrics [e.g. Shannon–Wiener diversity (DIV), richness (RICH)], and bacterial density (BAC), showed stronger relationships with catchment characteristics than hydrology (Table 2, Fig. S3). Riparian vegetation cover explained the largest part of the variance in the case of taxonomic diversity (DIV, VAR. PAR. RIP_VEG = 28%, Fig. S3) and taxonomic richness (RICH, VAR. PAR. RIP_VEG = 33%, Fig. S3), but only 13% for bacterial density within the sediment communities (Table 2 and Fig. S3). A small fraction of the variability was explained by

hydrological parameters, such as T_DRY, REW and T_DRYxREW, accounting for 5% of the diversity, 9% of the richness and 5% of the bacterial density (Fig. S3). Significant positive relationships were found between both the diversity and richness indices (DIV, RICH) and the riparian vegetation coverage (RIP_VEG, Fig. 3, Table 2). Specifically, linear models showed that abundant riparian vegetation determines a positive and significant increase in both the microbial diversity and richness indices, particularly at MD sites (Fig. 3A and B; Table 2). With regard to land-use, agricultural land cover explained the largest part of the bacterial density variance (BAC, VAR. PAR. AGRI = 33%) and it was also positively and significantly related to the bacterial density, but negatively related to community diversity (Table 2). The microbial composition of the sediments changed in response to the duration of the dry phase; sites with continuous flow had a significantly different community composition to that at intermittent sites [permutation analysis of variance (PERMANOVA), Fig. 4A]. This result was confirmed by the relation existing between NMDS1 and PC1 (Fig. 4B). In terms of taxonomy, the phylum Proteobacteria dominated the streambed communities at all sites, although its relative abundance decreased at intermittent sites (Fig. S4). Specifically, there was a significant reduction in the classes Delta-proteobacteria and Epsilon-proteobacteria between FL and LD sites [analysis of variance (ANOVA) $p < 0.05$; Fig. S4, Fig. 4A]. Similarly, the relative abundance of the phylum Planctomycetes decreased along the dry phase gradient, with a significant decrease in the relative abundance of the class Planctomycetia at MD and LD sites compared with FL sites (ANOVA $p < 0.05$; Fig. S4, Fig. 4A). In contrast, the relative abundance of the phyla Actinobacteria and Bacteroidetes doubled at sites with the longest dry phase (Fig. S4). More specifically, of the classes Actinobacteria ($r_s = 0.76$), Thermoleophilia ($r_s = 0.56$) and Sphingobacteria ($r_s = 0.65$) correlated strongly with the MD and LD communities (Fig. 4A); the relative abundance of Actinobacteria increased significantly at MD and LD sites (ANOVA, $p < 0.05$; Fig. S4, Fig. 4A), and Sphingobacteria at LD sites, compared with FL sites (ANOVA, $p < 0.05$; Fig. S4, Fig. 4A). The global correlation between the community composition and the functions was not significant ($p = 0.182$, $r_s = 0.10$), suggesting that these are uncoupled (Supplementary Information S4).

DISCUSSION

Overall, our findings confirm the hypothesis that microbial functions respond more strongly to hydrological factors than bacterial density and diversity do. The microbial community composition differs between flowing and intermittent sites whereas bacterial diversity and density respond to the environmental features of the catchment area, such as riparian vegetation cover and agricultural land use. Generally, reduced microbial activity, diversity changes and shifts in community composition are expected as the dry period lengthens (Rees et al., 2006; Amalfitano and Fazi, 2008; Marxsen et al., 2010), although co-occurring environmental factors are also often cited as important modulators for microbial responses to drought (Drenovsky et al., 2010; RuizGonzález et al., 2018). As previously reported, streambed sediments are completely exposed to the atmosphere under persistent dry conditions and may acquire characteristics similar to those of the adjacent soils (Steward et al., 2012; Morandi et al., 2014; Arce et al., 2019). A longer dry phase and modifications in the frequency of the dry–wet cycle in river ecosystems often determine the degree of similarity between streambed and terrestrial habitats (Harms and Grimm, 2012; Mori et al., 2017).

Drying gradient effects on streambed microbial function

Similar to previous studies, we found that the presence of specific extracellular enzyme activities (EEA) is influenced by the extended duration of the dry phase (Zoppini et al., 2014; Freixa et al., 2016; Gionchetta et al., 2019). With increasing dryness, PHE activity is enhanced indicating a greater capability to degrade lignin compounds, while the tendency of β -glucosidase and β -xylosidase to decrease suggested a reduced capability to degrade simple polysaccharides. This shift in enzyme capabilities suggests a gradual modification of the quality of organic matter available in streambed sediments when desiccating (Figs. 2 and 5, PHE, GLU and XYL). According to previous studies, reduced flow connectivity, accumulation of old plant litter and the development of terrestrial vegetation on the streambed (Battin et al., 2003; Fazi et al., 2013; Romaní et al., 2013) may enrich the sediment with lignin-like compounds. At the same time, desiccation may reduce the availability of simple polysaccharides due to the decreased input of freshly available organic compounds, such as those produced by algae in wet conditions (Acuña et al., 2007; Romaní et al., 2013; Freixa et al., 2016). Surprisingly, β -xylosidase activity was shown to be negatively related to riparian vegetation, even though this enzyme is usually involved in the degradation of hemicellulose

from plants (Romaní et al., 2013; Romaní et al., 2016). The large variability in enzyme activity observed between the intermittent sites may indicate a high level of plasticity in the microbial community, enabling them to select the source of organic matter. The overall expression of microbial enzyme activity is modulated by both the availability and the type of resources. For instance, as simple polysaccharides from algal activity and allochthonous plant materials from riparian vegetation are available, the production of the β -glucosidase enzyme would be favoured rather than β -xylosidase, since the end-product, glucose, is more energetically important than xylose. Nevertheless, the activity of β -xylosidase could be favoured when low riparian cover and scarce readily degradable algal compounds are present (Fig. 5). These results suggest that the coupled quantity and quality of organic matter influences microbial capacity to use organic materials, and they reveal adaptation to the sources available resulting from changes in intermittency and co-occurring factors, like riparian vegetation cover (Fig. 5). Long dry-phase sites are clearly characterized by the use of more terrestrial-origin materials (shown, in particular, by the increased PHE activity) despite presenting much less riparian vegetation (Table 2, Fig. 5). Besides the effect of the duration of the dry period, the occurring of wet episodes may enhance the microbial activity. This was observed in the case of respiration, which was particularly enhanced when prolonged rewetting occurred at hardly dry sites. In addition, the activity of the two hydrolytic enzymes varied greatly between the sites with short and intermediate length dry periods (SD and MD, Fig. S2). The large variability in these activities suggests that greater intermittency (i.e. alternation of dry and wet episodes) may determine larger fluctuations in microbial activity, which in turn could mask the expected decrease in β -glucosidase activity at the driest sites. Indeed, wet events make simpler and more labile compounds available, mobilizing these into the sediment (Birch and Griffiths, 1961; Fierer and Schimel, 2003; Rees et al., 2006). In this study, respiration was not measured directly after each wet event, but the highest values of β -glucosidase activity were observed in the frequently rewetted streambeds (MD sites), suggesting that the short wet events may reactivate a large number of dormant microbes (Timoner et al., 2012) or still-active oxidative enzymes from damaged or dead cells (Burns et al., 2013; Meisner et al., 2015).

Drying gradient effects on streambed community composition

The microbial community composition reflects clear differences in the relative abundance of certain taxa inhabiting the flowing and intermittent sites, suggesting that the presence of no flow episodes exerts an important effect, irrespective of the degree of intermittency. Microbes are capable of adapting rapidly to changing environmental conditions through structural and functional changes (Allison and Martiny, 2008; Wallenstein and Hall, 2012). Consequently, the stress provoked by a single dry period may determine the changes in intermittent microbial communities through the rapid osmotic adaptation of cells to cope with alternating dry and wet periods (Shade et al., 2012; Attermeyer et al., 2013; Meisner et al., 2018). As expected, in our study, increased dryness tended to modify the microbial communities' composition with respect to those of the flowing sites, involving higher relative abundances of drought-tolerant taxa and fewer drought sensitive taxa. This microbial community transition is characterized by a decrease in Gram-negative bacteria compared with an increase in the proportion of Gram-positive bacteria, which are more resistant and adapted to live in soil systems submitted to extreme drought (de Vries and Shade, 2013; Acosta-Martínez et al., 2014; Naylor, 2017). Furthermore, the microbial composition changes were driven by either just one or a few members of a microbial phylum, as also observed by Barnard and colleagues (2013) in soil systems. Specifically, the assemblage shifts in these streambeds included a significant decrease in the Gram-negative classes of Epsilon-proteobacteria and Deltaproteobacteria (phylum Proteobacteria) and Planctomycetia (phylum Planctomycetes). Conversely, we found significant increases in the Gram-positive classes Actinobacteria and Thermoleophilia (phylum Actinobacteria) as observed in extremely desiccated soils (Bouskill et al., 2013). The spread of the class Sphingobacteria (phylum Bacteroidetes) in the driest intermittent communities could be due to rewetting effects (last rewetting event prior to sampling). Sharp increases in the relative abundance of the class of Sphingobacteria have often been associated with abrupt wet events in arid and desert soil communities (Aslam et al., 2016; Meisner et al., 2018). In contrast to community composition, richness and diversity were not significantly modified by hydrological history, suggesting that there was taxa-dominance alternation or substitution without increased dryness significantly affecting the overall diversity. This suggests that desiccation may also play a role in decreasing diversity and richness with a concomitant decrease in riparian vegetation cover.

Relevance of environmental catchment characteristics

Riparian vegetation and land use (agricultural cover) were significant drivers of streambed microbial community richness and diversity. While riparian vegetation positively influenced the diversity of the microbial community, agricultural cover negatively affected bacterial diversity but enhanced bacterial density. Riparian vegetation may help conserve stream biodiversity and ecosystem functioning (Elosegi et al., 2010). The greater bacterial diversity and richness associated with the greater riparian vegetation cover could be related to more microhabitats available for microbes (e.g. through leaf fall, pool persistence, colonization by terrestrial plants) (Romaní et al., 2017; Arce et al., 2019). On the other hand, although we found no direct effect of hydrological history on microbial diversity in our study, reduced riparian vegetation co-occurred with longer dry periods. With regard to land use, agricultural cover was the major force driving reduced streambed bacterial diversity while at the same time increasing bacterial density. Even though most of the sites studied presented similar catchment characteristics, some had important agricultural field cover in their surroundings. These microbial community responses might be a consequence of enhanced nutrient input from agricultural practices in the fields around the sites; this may induce bacterial growth and favour the spread of certain dominant species, thus reducing diversity, as expected in eutrophic conditions or nutrient-contaminated systems (Zeglin, 2015; Li et al., 2018). The negative effect of agricultural cover on bacterial diversity may be further enhanced by longer droughts (negative interaction T-DRYxAGRI, Table 2), as also suggested in previous studies on leaf litter decomposition and soils (Duarte et al., 2017; Meisner et al., 2018).

CONCLUDING REMARKS

Considering both microbial function and the response of streambed community composition, these findings highlight the fact that hydrological history has a significant effect although functional, composition and diversity responses did not occur in parallel (absence of correlation in Mantel test). Interestingly, the response of function, in terms of organic matter use capability (i.e. extracellular enzymes) presented a gradient with increased use of PHE (related to lignin compounds) and decreased use of β -glucosidase and β -xylosidase (linked to simple polysaccharides degradation) in streambeds suffering longer dry periods, as well as a

high degree of variability at intermittent sites. In contrast, streambed community composition differed between sites with flowing waters and intermittent conditions, irrespective of the drying intensity, while richness and diversity were strongly modulated by the characteristics of the catchment area. Contradictory results are also reported in the literature, where some studies present coupled compositional, diversity and functional microbial responses to water stress, while others show activity responses to desiccation decoupled from changes in microbial community diversity and/or composition (Frossard et al., 2012; Pohlen et al., 2013; Febria et al., 2015; Zoppini et al., 2016). The intrinsically high variability of a field assessment could explain the unexpectedly weak correlation observed between the microbial community composition and functions, bearing in mind the fact that the activities and diversity measured in this work do not exhaustively explain the entire system. Overall, the maintenance of microbial diversity under extreme dry streambed conditions potentially ensures reduced losses in system functioning. Nevertheless, further studies are needed to confirm whether longer dry periods combined with reduced riparian vegetation and increased agricultural land cover could threaten microbially mediated processes linked to the carbon cycle.

EXPERIMENTAL PROCEDURES

Study area, site selection and sampling strategy

A total of 37 upstream sites were selected and surveyed in streams situated in the northeastern Iberian Peninsula (Catalonia). These sites belong to nine watersheds corresponding to the main rivers in that area, flowing into the Mediterranean Sea, i.e. the Muga, Fluvià, Ter, Tordera, Llobregat, Francolí, Foix, Besós and Ebro rivers (Fig. S1). The sampling sites include lowland and midmountain watercourses (altitude 80% totally shaded, considered length 100 m) and riparian vegetation cover (e.g. the presence of trees, shrubs or small bushes was considered as a percentage, from 75%, for the first 10 m width of riparian vegetation) were measured in situ following a field assessment procedure. Once at the laboratory, sediment subsamples of about 1 ml volume (1 cm² projected surface area) were created and collected from each sediment corer, using an uncapped syringe, for EEA, respiration, bacterial community composition and density analyses. Community respiration and EEA were

measured within 24 h of sampling. Bacterial density samples were fixed with 10 ml of filtersterilized (0.2 μ m pore size nylon filters Whatman, Kent, UK) synthetic water (13 mg L⁻¹ Na₂SO₄, 16.1 mg L⁻¹ Na₂SiO₃, 29.4 mg L⁻¹ CaCl₂*2H₂O, 0.6 mg L⁻¹ KCl, 3 mg L⁻¹ MgSO₄*7H₂O, 26.5 mg L⁻¹ Na₂CO₃, 0.6 mg L⁻¹ NH₄H₂PO₄ and 7.3 mg L⁻¹ (NH₄) (NO₃) in MQwater) and formaldehyde (2% final concentration), and then stored at room temperature. For the microbial diversity analysis of the 16S rRNA gene, the sediments were stored at -20C. The remaining sediment was used to determine organic matter and water content.

Hydrological history and catchment characteristics of the study sites

One year prior to the sampling, temperature and pressure transducers (Solinst Levellogger Gold Model 3001 and Solinst Barologger Gold Model 3001, Solinst, Georgetown, ON, Canada), and temperature data loggers (ACR SmartButton Logger, MicroDAQ) were installed at the study sites to characterize their hydrology. All the temperature loggers recorded data every 60 min. Once the entire dataset for each sampling site had been obtained, the daily variations in the streambed and air temperature were estimated. To characterize the stream hydrology in the intermittent stream, we used the daily variation of the streambed temperature corrected for the barometric pressure and air temperature. This daily variation was determined as the difference between the maximum and minimum temperature per day and the daily higher rate of change per hour (Colls et al., 2019) (see Supplementary Information S1, Arias-Real et al., 2018). In addition, the physical and chemical characteristics of the sites were recorded (Supplementary Information S2). In total, the hydrological data collected covered an 8 month period (245 days) of hydrology history for each stream site, and the related parameters calculated from this database were: the total number of dry days in the 8-month period (T_DRY); the percentage of dry days in the 8-month period (P_DRY); the duration (days) of the last dry event prior to the sampling (L_DRY); the duration of the last rewetting period (i.e. the number of consecutive wet days prior to the sampling) (REW) and the number of dry/rewetting cycles over the 8-month period (FREQ). As well as hydrological descriptors, in situ data corresponding to sediment organic matter, water content, shade cover and riparian vegetation were collected, and land use in the catchment area of the sites was determined. The organic matter content was measured as AFDW (Ash-Free Dry Weight) and 1 ml of sediment

sample was dried at 70C for 72 h and then burnt for 4 h at 450C using a muffle furnace (AAF 1100, Carbolite, UK). The results were expressed as grams of AFDW per gram of sediment dry weight (DW). In order to estimate the sediment saturation, sediment water content was calculated as the percentage of water loss (% difference) between the fresh and DW (1 ml for each sample). Major land covers (urban zones, agricultural and forested areas, Corine Land Cover 2012, <https://www.eea.europa.eu/data-and-maps/data/clc-2012-raster>) were calculated as percentage considering the adjacent 1 km upstream of the sampling point, within the catchment area.

Microbial response variables

Bacterial density and community respiration. Bacterial density in sediments was determined through flow cytometry (FACSCalibur, Becton Dickinson), following a protocol adapted from Amalfitano and Fazi (2008). Sediment samples were sonicated for 1 min, shaken for 30 s, and sonicated again for 1 min to extract the biofilm from the sediment grains (Ultrasons, Selecta). A subsample of the obtained extract (1 ml) was pipetted into a glass vial and 9 ml of detaching solution (distilled water, NaCl at 0.85% final concentration, Tween 20 at 0.5% final concentration and sodium pyrophosphate at 0.1 M, final concentration) was added to promote the separation of the cells. The samples were shaken for 30 min (150 rpm, in the dark and at room temperature), left for 10 min at 4C, and then sonicated with ice for two 1 min cycles. Next, the samples were shaken again for 1 min and left for 5 min, to allow the sedimentation of the larger particles, and, finally, 1 ml of supernatant was transferred into a clean sterile Eppendorf tube. Once the sediment extracts had been obtained, each sample was purified through Nycodenz (1 ml), added to the bottom of each Eppendorf tube, and centrifuged (14 000 rpm) for 90 min at 4C. The purified sediment extracts (400 µl) were stained with Syto13 (4 µl Fisher, 5 µM solution) and incubated in the dark for 30 min. The stained samples were counted using flow cytometry (FACSCalibur, Becton Dickinson). To normalize fluorescence data, a known concentration of bead solution (10 µl of 10⁶ beads ml⁻¹, Fisher 1.0 µm) was added to the samples. The bacterial density (BAC) results were expressed as cells × 10⁸ per gram of sediment DW. Resazurin assay was applied to determine community respiration (RESP), using a modified version of the technique in Haggerty and colleagues (2010). The resazurin (7-Hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt, RAZ) method consists of

using a redox dye (blue in the oxidized state) that indicates the respiratory activity of microorganisms (it turns pink when reduced). Before applying the resazurin dye, we prepared the sediment extract for each sample replicate from 1 ml of sediment, placed in a sterile vial with 5 ml of pyrophosphate solution 50 mM (Manini and Danovaro, 2006). Each sample was sonicated for 1 min in ice in order to preserve the cell structure (Amalfitano and Fazi, 2008), shaken for 30 s, and sonicated again for 1 min to extract the biofilm from the sediment grains (Ultrasons, Selecta). One milliliter subsamples of the extract was diluted in 9 ml of filtered water (0.2 μ m pore size nylon filters Whatman, Kent, UK). Aliquots of each sediment extract (166 μ l) were placed in a transparent microplate (MicroWell™ Nunc™, ThermoFisher Scientific) and mixed with ringer solution (28 μ l) and resazurin stock substrate (6 μ l of 0.35 mM Sigma-Aldrich, USA). The plate was closed with a lid, then incubated (target temperature 20C), and each cycle started with shaking for 90 s (shaking amplitude 3 mm), with a pause of 10 s before each absorbance reading. The incubation period lasted 24 h and the absorbance was read every 4 min (603 and 570 nm resazurin and resorufin wavelengths). Subsequently, we used the standard resorufin (pink compound that indicates the conversion of resazurin to resorufin) curve and the absorbance values obtained after 18 h of incubation to define the community respiration as micromoles of resazurin respired per grams of DW h⁻¹ (μ mol RAZ gDW⁻¹ h⁻¹).

Extracellular enzyme activities

Fresh sediment subsamples (1 ml) were analysed for the activity of two hydrolytic enzymes, β -D-1, 4-glucosidase (EC 3.2.1.21, GLU), β -xylosidase (EC 3.2.1.37, XYL), as well as for the oxidative enzyme PHE (EC 1.14.18.1, PHE), as indicators of the cellulose, hemicellulose and lignin compound decomposition capacity respectively. For the EEA, we followed the procedure from Gionchetta and colleagues (2019). The hydrolytic enzyme (GLU and XYL) values were expressed as nmol MUF gDW⁻¹ h⁻¹, and the PHE enzyme values were expressed as nmol 2,3-dihydroindole-5,6-quinone-2-carboxilate (DIQC) gDW⁻¹ h⁻¹.

Community composition and diversity

DNA extraction was performed from 0.5 g of sediment for each composite sample (n = 5 for each stream site) using the FastDNA™Spin Kit for Soils (MP Biomedicals, Irvine, CA)

according to the manufacturer's instructions. The DNA concentration and quality in each sample were measured spectrophotometrically (Nanodrop2000, Thermo Scientific™, Waltham, USA) and further assessed with PCR (95C × 5 min; 35 cycles: 95C × 1 min; 55C × 1 min; 72C × 1 min; 72C × 10 min) and 1% agarose gel electrophoresis. The DNA extracts were stored at −20 °C until sequencing analysis at the Roy J. Carver Biotechnology Center (University of Illinois, IL, USA). The diversity of prokaryotes (including bacteria and archaea) was analysed utilizing the primer pair V4_515F/V4_806R61 (Caporaso et al., 2011), targeting the V4 region of the 16S rRNA gene, using Illumina MiSeq technology. We analysed both bacteria and archaea species in sediments, but the latter was excluded from the analysis due to extremely low read values. IM-TORNADO pipeline version 2.0.3.2 (Jeraldo et al., 2014) was used to generate the operational taxonomic unit (OTU) table and assign taxonomy to the corresponding OTUs (≥97% sequence similarity). Bacterial taxonomy was assigned through the Ribosomal Database Project (RDP version 10, Cole et al., 2014). Diversity analyses were performed using the IMTORNADO generated output BIOM table. The range of sequencing depth was between 9668 and 127 452 of DNA for total prokaryotes. OTU tables were generated from the BIOM table using the phyloseq package for analysing microbiome data, by R software (R version 3.4.1). Rarefaction curves were generated (rarefy_even_depth function, phyloseq package) and the rarefied OTU table was set to the smallest count of bacterial reads (9211 reads/sample) in order to minimize bias due to different numbers of total sequences present in the samples. Two alpha-diversity metrics were calculated from the OTU table, i.e. the Shannon–Wiener index (DIV) and the Chao1 richness index (RICH), using the phyloseq package by R software (R version 3.4.1). The raw sequence dataset was deposited in the NCBI Sequence Read Archive database under Accession No. PRJNA557375.

Data analyses

To summarize the hydrological history and catchment characteristics, and assess the existence of gradients between the study sites and key driving factors, we performed a PCA including all the parameters analysed (total number of dry days in the 8-month period, T_DRY; the duration (days) of the last dry event prior to the sampling, L_DRY; the number of consecutive

wet days prior to the sampling, REW; frequency of dry/rewetting cycles in the 8-month period, FREQ; sediment water content, WAT_CON; organic matter content, ORG_MAT; riparian vegetation cover, RIP_VEG; shade cover, SHAD; forested land use, FORE; agricultural land use, AGRI; urban land use, URBA). Although the hydrological history was mainly studied as a continuous factor, in order to allow some general analyses, the streambed sites were classified according to the duration of their dry phase in a similar way to the proposal of Costigan et al., (2017), and four classes were defined for our study sites: flowing sites (FL, continuous flow); short-dry phase sites (SD, from 1% to 15% dry days); medium-dry phase sites (MD, 15%–85% dry days over the 8-month period); and longdry phase sites (LD, average 85%–100% dry days) (Fig. S1, Table 1). The differences between the hydrological categories (FL, SD, MD and LD sites) were analysed for EEA, community respiration, bacterial density and microbial diversity and richness indices, using one-way ANOVA. To analyse the response of microbial functions (extracellular enzymes and respiration), and diversity and bacterial density, to the hydrological and catchment characteristics, we followed the multiple-predictor analytical procedure using survey data (see Feld et al., 2016). First, we ran exploratory analyses to reduce skewness when necessary: GLU (β -glucosidase activity) and XYL (β -xylosidase activity) were square-root transformed; PHE (PHE activity), RESP (community respiration), BAC (bacterial density), DIV (Shannon diversity index), RICH (Chao1 richness index) and RIP (riparian vegetation) were log-transformed). Consequently, we standardized and checked the collinearity between the variables (Feld et al., 2016). Second, using Random Forest (RF) analysis (package ForestSRC, R software) (Ishwaran et al., 2014) we ranked, identified and visualized all the predictor interactions for each response variable: hydrological history parameters (T_DRY, L_DRY, REW, FREQ); sediment water and organic matter (WAT_CON, ORG_MAT); shade cover (SHAD); riparian vegetation (RIP_VEG); and agricultural (AGRI), forested (FORE), and urban (URBA) land use. The number of trees in the RF was 3000 and the variables used in each split was set to three (Feld et al., 2016). Afterwards, we applied generalized linear models to model the response of each variable to single and combined predictors (Feld et al., 2016; Grueber et al., 2011; Barton, 2019). For each model, we used a maximum of four predictors, selected considering the RFs (Feld et al., 2016), including two hydrological predictor terms (T_DRY and REW), and at least two environmental predictors for each

response variable. Subsequently, the multi-model averaging approach (MuMIn R package) was applied. This procedure generated all possible models and ranked them based on model weight < 0.05 for all tests. Details on the analytical procedure are presented in Supplementary Information S3. The differences in microbial community composition between the study sites were analysed using non-metric multidimensional scaling (NMDS) ordination, based on Bray–Curtis dissimilarity performed at the OTU taxonomic level. The OTU relative abundance matrix used for the ordination analyses was previously fourth root transformed. A PERMANOVA (9999 permutations) was applied to test the significant differences in the community composition between the four hydrological history categories (PRIMER 6 version 6.1.11 and PERMANOVA+ version 1.0.1). In order to identify the taxonomic classes significantly related to the OTU distribution, specific vector fitting analysis was applied to the NMDS (Legendre and Legendre, 1998). A further one-way ANOVA was applied to determine significant differences between the relative abundances of the taxonomic classes inhabiting the different hydrological history categories (FL, SD, MD and LD sites). Finally, a Mantel test was performed between community composition and the functional matrices to assess whether they responded similarly to flow intermittency (Bray–Curtis dissimilarity for the compositional matrix and Euclidian distance for the functional data, and then RELATE analysis with Spearman rank correlation in Supplementary Information S4, PRIMER 6 version 6.1.11 and PERMANOVA+ version 1.0.1).

ACKNOWLEDGEMENTS

We gratefully acknowledge Veronica Granados and Miriam Colls for assistance in the field, and Jordi Bou Manobens for his help with the creation of the GIS map. Special thanks go to the team at Laboratoire Microorganismes: Génome et Environnement for their assistance in the laboratory, and to Cayetano Gutierrez-Canovas for his help with the statistical analyses. Giulia Gionchetta acknowledges the UdG fellow- ship (IFUdG 2016) from the University of Girona. This study was supported by the Spanish Ministry of Economy and Competitiveness through the project FUNSTREAM (CGL2014-58760-C3-R), by the Economy and Knowledge Department of the Catalan Government, through Grant/ Award Number: 2014 SGR 484, by the project DryHarshSal (RTI2018-097950-B-C21) of the Spanish Ministry of

Science, Innovation and Universities, and through the Short-Term Scientific Mission (STSM) Grant awarded by the Science and Management of Intermittent Rivers and Ephemeral Streams (SMIRES) Cost-Action (Action number: 40271). We are grateful to the two anonymous reviewers that helped improve the initial version of the manuscript.

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FIGURE LEGENDS

Fig. 1. Principal component analysis (PCA) showing the importance of the environmental and hydrological factors that best distinguished our 37 study sites, represented by arrows. The colours and shape of each site define the corresponding hydrological group (legend). [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 2. Responses of the extracellular enzymatic activities (GLU, β -glucosidase; XYL, β -xylosidase; and PHE, phenoloxidase) and community respiration (RESP) to the hydrology (x-axis, total dry days, T_DRY) and the duration of the last wet period (REW) using linear models. The wet duration was fixed at three levels: the 5% (low), 50% (mean) and 95% (high) percentiles. The predictor selection and interaction for each model were based on the Random Forest analyses (Table 2). The colours of each site define the corresponding hydrological group (legend). [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 3. Response of the microbial taxonomic diversity (DIV, Shannon-Wiener index) and richness (RICH, Chao1 index) to the hydrology (x-axis, total dry days, T_DRY) and the abundance of riparian vegetation (RIP_VEG) using linear models. The abundance of riparian vegetation was fixed at three levels: the 5% (low), 50% (mean) and 95% (high) percentiles. The predictor selection and interaction for each model were based on the Random Forest analyses (Table 2). The colours of each site define the corresponding hydrological group (legend). [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 4. A. NMDS ordination plot representing the bacterial community composition (OTUs

abundance) at the study sites (coloured according to hydrological category, legend). The arrows indicate correlations with taxonomic classes (Spearman coefficient $r_s > 0.6$). Taxa in bold portray significant differences between FL and MD/LD sites (ANOVA $p < 0.05$). The PERMANOVA test of the NMDS ordination analysis and subsequent pairwise comparisons indicated significant differences between hydrological categories ($p = 0.003$) where the flow site communities were significantly different to the three other intermittent bacterial communities ($p = 0.051$, $p = 0.001$, $p = 0.001$ for the FL-SD, FL-MD, and FL-LD comparisons respectively). There were no significant differences found between the three intermittent categories ($p > 0.1$ for all comparisons). B. Relationship between NMDS1 (community composition) and PC1 (hydrological sites distribution). The adjusted regression coefficient is 0.54. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 5. Conceptual diagram summarizing the main results obtained for the intermittent streams studied. The quantity and quality of the organic matter (OM) and the abundance of riparian vegetation (RIP_VEG.) apparently drove the selection of specific degradation enzymes (such as hydrolytic β -xylosidase, XYL, or the recalcitrant activity of phenol-oxidase, PHE). The shifts in bacterial community composition towards drought-resistant taxa intensify under conditions of prolonged desiccation, as illustrated by the circles (the violet and grey refer to Gram-positive and negative bacteria respectively). [Color figure can be viewed at wileyonlinelibrary.com]

Table 1. The average values (mean) and standard errors (SE) of the hydrological and environmental characteristics measured for each group of sampling sites: FL, flow sites; SD, short dry-phase sites; MD, medium dry-phase sites; LD, long dry-phase sites.

	FL		SD		MD		LD	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T_DRY (no. of total dry days)	0.00 ^a	±0.00	18.57 ^a	±4.67	104.1 ^b	±10.22	224.7 ^b	±8.61
L_DRY (no. of last dry days)	0.00 ^a	±0.00	7.00 ^a	±2.68	28.75 ^a	±9.61	122.0 ^b	±40.79
REW (no. of last wet days)	245.0 ^a	±0.00	79.86 ^b	±17.97	29.31 ^c	±5.26	5.29 ^c	±2.55
FREQ (no. of dry/wet cycles)	0.00 ^a	±0.00	3.43 ^{ab}	±0.48	4.85 ^b	±0.71	2.71 ^{ab}	±0.52
ORG_MAT (g DW ⁻¹)	0.01 ^a	±0.00	0.02 ^{ab}	±0.01	0.02 ^{ab}	±0.00	0.03 ^b	±0.01
WAT_CON (%)	15.15 ^a	±2.69	13.31 ^a	±2.62	15.38 ^a	±1.62	15.74 ^a	±6.09
BAC (cells × 10 ⁹ gDW ⁻¹)	2.53 ^a	±0.78	5.56 ^a	±2.66	8.14 ^a	±4.90	4.18 ^a	±1.47
SHAD (%)	56.43 ^a	±9.80	46.43 ^a	±9.68	46.47 ^a	±5.55	30.71 ^a	±8.96
RIP_VEG (%)	80.71 ^a	±9.09	55.01 ^{ab}	±9.88	42.14 ^{ab}	±6.04	32.14 ^b	±8.99
AGRI (%)	16.17 ^a	±13.81	25.53 ^a	±15.95	25.35 ^a	±9.42	17.32 ^a	±13.66
FORE (%)	83.59 ^a	±13.79	74.22 ^a	±15.89	67.73 ^a	±9.24	77.73 ^a	±13.05
URBA (%)	0.23 ^a	±0.20	0.24 ^a	±0.16	6.92 ^a	±3.45	4.96 ^a	±2.54

The letters indicate the significance obtained from the ANOVA post hoc comparisons (Tukey test). Predictors considered: total dry phase duration (T_DRY, the total number of dry days over the 8-months); duration of the last dry period prior to the sampling (L_DRY); duration of the last wet period prior to the sampling (REW); the frequency of dry/rewetting cycles over the 8 months (FREQ); organic matter content (ORG_MAT); sediment water content (WAT_CON); shade cover (SHAD); riparian vegetation cover (RIP_VEG); forested land use (FORE); agricultural land use (AGRI); urban land use (URBA).

Table 2. Results of the linear models created for each microbial functional and structural response variable.

	SES	SE	p value	mod.rel.imp.	r ² (%)
GLU					
ORG_MAT	0.633	0.225	0.007**	1.00	0.31
WAT_CON	-0.043	0.196	0.831	0.12	0.31
T_DRY	-0.553	0.551	0.332	0.57	0.31
REW	-0.591	0.647	0.376	0.79	0.31
T_DRY*REW	-0.331	1.069	0.766	0.13	0.31
XYL					
RIP_VEG	-0.609	0.177	0.001***	1.00	0.46
ORG_MAT	0.687	0.181	0.001***	1.00	0.46
T_DRY	-0.496	0.209	0.022 *	1.00	0.46
REW	0.042	0.234	0.861	0.27	0.46
T_DRY*REW	na	na	na	na	0.46
PHE					
WAT_CON	0.951	0.585	0.117	0.60	0.21
T_DRY	1.621	0.769	0.041*	1.00	0.21
REW	0.813	0.882	0.375	0.37	0.21
T_DRY*REW	na	na	na	na	0.21
RESP					
ORG_MAT	0.286	0.339	0.421	0.22	0.17
T_DRY	2.022	1.834	0.282	0.44	0.17
REW	2.015	2.737	0.471	0.47	0.17
T_DRY*REW	3.261	1.746	0.075.	0.27	0.17
DIV					
AGRI	-0.227	0.082	0.004**	1.00	0.53
RIP_VEG	0.285	0.091	0.001***	1.00	0.53
T_DRY	-0.015	0.241	0.690	1.00	0.53
REW	-0.135	0.342	0.599	1.00	0.53
T_DRY*AGRI	-0.229	0.089	0.014*	1.00	0.53
T_DRY*REW	0.109	0.428	0.809	0.28	0.53
RICH					
FORE	1.731	2.804	0.556	0.23	0.38
RIP_VEG	355.15	97.92	0.001***	1.00	0.38
T_DRY	306.00	391.42	0.448	0.25	0.38
REW	128.75	525.14	0.810	0.47	0.38
T_DRY*REW	679.23	446.93	0.148	0.13	0.38
BAC					
AGRI	0.791	0.176	0.001***	1.00	0.49
RIP_VEG	-0.439	0.200	0.034*	1.00	0.49
T_DRY	-0.092	0.227	0.695	0.48	0.49
REW	0.010	0.014	0.484	0.29	0.49
T_DRY*AGRI	0.299	0.210	0.171	0.32	0.49
T_DRY*REW	0.017	0.010	0.112	0.15	0.49

These models examined the effect of total dry days (T_DRY), the rewetting duration prior to the sampling (REW), their interaction (T_DRY*REW) and at least two environmental predictors. The predictor set for each model was selected considering the results of the Random Forest analyses. Predictors and interaction standardized effect size (SES), standard error (SE) and significance (p value) for each model are shown (significant values are indicated in bold and by: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1). The na interaction values indicate unavailable results that were automatically excluded from the model as their importance was too low. The relative predictor importance of the model (mod.rel.imp.) and the goodness of-fit of the model (r²) are also specified for each predictor. The microbial functional and structural variables considered for modelling were: β -glucosidase, GLU; β -xylosidase, XYL; phenol-oxidase, PHE; community respiration, RESP; Shannon diversity

index, DIV; richness index, RICH; bacterial density, BAC.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

964 **Supplementary information:**

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Supplementary information S1: Detailed hydrological measurements

Daily variation in streambed and air temperatures were used to identify, for each sampling site, the presence of water in the streambed, which is strongly related to its temperature daily variation (Constantz et al., 2001; Stromberg et al., 2005). The temperature transducers (Solinst Levellogger Gold Model 3001, Solinst Ltd, Georgetown, ON, Canada) and temperature data loggers (ACR SmartButton Logger, MicroDAQ) used for the hydrological characterization were allocated in all streams, on the bottom of riffles areas. Furthermore, the air temperature was obtained from the data loggers (Solinst Barologger Gold Model 3001, Solinst Ltd, Georgetown, ON, Canada) previously installed in the riparian zone of each stream site, or obtained from nearby meteorological stations (Servei Meteorologic de Catalunya; <http://www.meteo.cat/>). All the applied temperature loggers recorded data every 60 minutes. Once obtained the entire dataset for each sampling site the daily variations in streambeds and air temperatures were estimated by two ratios as presented in Colls et al., (2019).

Once data had been obtained, the daily variations in streambed and air temperatures were characterized by two ratios: the daily streambed-to air temperature amplitude ratio (DA) and the streambed-to-air temperature change rate ratio (that is, heating or cooling; RTC). Both ratios were based on the relationship between the daily variations in streambed and air temperature. To reinforce results, each one assessed a different aspect of the temperature oscillations. DA was determined by the difference between the maximum (max(T)) and the minimum (min(T)) temperatures in the streambed and the air during a whole day. RTC was determined as the ratio between the highest hourly temperature change rate in the streambed and the air, in each sampling site.

A moving average of the DA and RTC ratios was calculated, comprising a total of 5 days to overcome potential erroneous interpretations of the stream's flow or no-flow state. This allowed dampening extreme values and achieving the best fit to water level data. The output value was named hydrological status (HS). Starting from the HS serial datasets we calculated all the hydrological parameters for each streambed.

For more specific information about the hydrological characterization and to visualize the DA, RTC and HS equations we referred to Colls et al. (2019) and Arias-Real et al., (2018), being part of the same multi-sites project.

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1017 **Supplementary information S2: physical-chemical streams characteristics**

Stream	Stream code	GPS coordinates	DOC	TN	SRP	NH4+	Ca2+	K+	Si+	Na+	Mg2+	Mn2+	Fe2+	Cl-	NO2-	NO3-	SO42-
ppm																	
Llobregat	LL3	41°41'43.67"N	3.61	0.15	0	0.04	88.51	2.19	2.47	22.85	62.42	<0.1	0.05	36.98	<0.1	0.09	144.5
		1°53'33.00"E															
	LL5	41°41'46.46"N	2.05	0.31	0.01	0.01	77.52	0.73	2.49	8.64	27.64	<0.1	<0.1	21.65	<0.1	0.99	11.16
		1°59'1.67"E															
		41°46'1.10"N	3.99	0.14	0.01	0.07	48.15	1.38	8.75	15.71	16.89	<0.1	<0.1	7.62	<0.1	0.23	77.37
	BE7	41°47'35.79"N	4.99	0.22	0.02	0.06	47.05	1.32	5.87	12.11	11.88	<0.1	<0.1	8.58	<0.1	0.15	23.51
		2°17'29.77"E															
		41°25'7.63"N	6.64	8.7	0.23	0.07	160.8	5.68	5.22	6.55	29.23	<0.1	<0.1	17.53	0.23	11.44	188.1
	FO11	1°30'26.44"E															
		41°23'52.63"N	1.51	0.71	0.01	0.07	162	2.15	4.28	12.11	55.01	<0.1	<0.2	20.71	<0.1	2.01	340.7
		1°35'37.29"E															
	TO13	41°51'55.61"N	1.66	0.58	0.01	0.05	51.2	1.16	10.94	17.94	9.27	<0.1	<0.1	12.93	<0.1	1.68	9.95
		2°35'35.62"E															
		41°41'40.88"N	2.05	1.56	0.18	0.05	33.62	2.22	6.67	21.9	9.91	<0.1	<0.2	19.93	<0.1	3.58	15.29
	TO14	2°29'0.21"E															
Ter	TE17	42°5'20.88"N	0.66	0.55	0.02	0.07	103.7	1.49	5.71	7.34	17.1	<0.1	<0.1	7.24	<0.1	1.6	7.83
	TE18	2°35'17.97"E															
		42°7'17.10"N	2.87	0.49	0.01	0.01	77.68	2.15	2.46	4.66	20.32	<0.1	<0.1	8.68	<0.1	1.49	31.12
	TE19	2°13'11.86"E															
		42°2'52.10"N	2.71	0.51	0.06	0.09	109.5	3.53	4.22	9.51	22.56	<0.1	<0.1	10.02	<0.1	1.05	26.15
	TE20	2°24'38.68"E															
		42°4'18.76"N	0.94	2.31	0.01	0.07	109.6	1.8	6.17	7.37	19.95	<0.1	<0.1	8.98	<0.1	5.54	11.25
	TE21	2°32'28.68"E															
		42°4'39.68"N	3.83	0.61	0.01	0.08	80.72	2.09	1.99	9.13	16.36	<0.1	<0.1	8.8	0.18	1.62	38.79
	TE22	2°20'19.50"E															
		42°10'25.26"N	3.25	0.13	0.01	0.07	127.9	2.35	2.13	7.06	28.11	<0.1	<0.1	3.01	0.18	0.02	139.9
Francoli	FR27	2°10'21.70"E															
		41°59'14.24"N	3.03	1.24	0.03	0.07	121.5	3.59	6.55	45.93	25.95	<0.1	<0.1	20.84	0.18	3.31	26.82
		2°50'15.19"E															
		42°6'35.03"N	3.6	0.26	0.02	0.01	116.5	2	2.66	11.75	13.3	<0.1	<0.1	17.24	0.18	0.49	41.44
	TE25	2°29'20.27"E															
		41°55'15.45"N	2.85	0.52	0.08	0.01	71.3	2.04	5.34	54.92	15.74	<0.1	<0.1	67.86	0.18	1.25	22.69
	TE26	2°42'41.04"E															
		41°18'38.37"N	1.15	0.22	0.01	0.06	100	1.03	3.55	5.31	38.43	<0.0	<0.1	8.76	<0.1	0.76	123.7
		1°5'2.51"E															
		42°7'37.99"N	3.52	2.63	0.29	0.01	141.3	4.24	5.09	18.69	20.78	<0.0	<0.1	13.44	<0.1	5.38	38.89
Fluvia	FL32	2°38'26.65"E															
		42°7'28.45"N	6.32	0.93	0.03	0.01	69.07	1.76	2.38	9.41	14.12	<0.0	<0.1	14.42	<0.1	3.54	37.72
	FL33	2°26'29.72"E															
		42°6'51.11"N	7.02	1.37	0.08	0.03	69.99	2.72	2.38	5	10.78	<0.1	<0.1	10.07	0.18	4.99	21.99
	FL34	2°26'53.48"E															
		42°19'2.62"N	1.99	0.2	0.01	0.07	97.62	1	3.87	4.73	15.27	<0.1	<0.1	3.91	<0.1	0.75	55.37
	MU36	2°42'13.25"E															
		42°23'15.61"N	4.02	0.55	0.04	0.08	38.14	1.15	9.53	30.23	12.62	<0.1	<0.1	25.29	0.18	0.96	55.26
	MU37	3°3'6.24"E															
		42°23'6.91"N	4.26	0.87	0.02	0.07	28.02	2.76	7.44	16.96	7.98	<0.1	<0.1	11.57	0.18	2.03	37.95
	MU38	3°1'59.28"E															
Ebro	EB40	40°59'51.89"N	3.3	0.41	0.01	0.06	192.4	4.02	4.88	132.1	172.7	<0.1	<0.1	208.8	<0.1	0.84	477.4
		0°8'5.54"E															
	EB42	41°15'24.80"N	1.87	0.2	0.01	0.07	91.03	1.24	3.22	5.77	46.3	<0.1	<0.1	7.15	0.18	0.37	100.3
		0°56'34.58"E															
	EB43	41°12'14.84"N	2.77	4.3	0.02	0.13	297.9	7.4	3.02	47.57	120.2	<0.1	<0.1	29.43	<0.1	6.32	586
		0°14'57.05"E															
	EB44	40°52'11.42"N	0.89	0.78	0.01	0.06	70.33	1.03	2.64	3.53	28.08	<0.1	<0.1	4.5	<0.1	1.76	13.24
		0°9'36.60"E															
	EB45	40°58'48.19"N	2.05	5.45	0.07	0.05	305.5	3.67	5.25	21.98	64.03	<0.1	<0.1	23.88	<0.1	8.53	488.1
		0°24'24.60"E															
	EB48	41°0'5.81"N	1.47	0.28	0.01	0.09	217.1	0.96	4.31	21.52	62.58	<0.1	<0.1	23.88	<0.1	0.7	391.7
		0°23'4.08"E															
	EB49	41°0'35.87"N	4.88	0.6	0.02	0.13	511.9	17.2	4.52	163.1	245.1	<0.1	<0.1	59.78	<0.1	0.15	1426
		0°23'31.90"E															

1019 Component analysed: Dissolved organic carbon, DOC; Total nitrogen, TN; Soluble reactive phosphorous, SRP; Ammonium,
1020 NH_4^+ ; Calcium, Ca^{2+} ; Potassium, K^+ ; Silicon, Si^{4+} ; Sodium, Na^+ ; Magnesium, Mg^{2+} ; Manganese, Mn^{2+} ; Iron, Fe^{2+} ; Chlorine, Cl^- ;
1021 Nitrite, NO_2^- ; Nitrate, NO_3^- ; Sulphate, SO_4^{2-} .

1022 **Supplementary information S3: Details of the modelling procedure**

1023 Specifically, the procedure followed consisted in 4 main steps:

1024
1025 The first step is to ensure the requirements for data quality and consistency for the subsequent
1026 analyses. Once the data transformation and standardisation was assessed we proceed addressing the
1027 collinearity through the Variance Inflation Factor (VIF), which is a way to quantify collinearity. This
1028 method also accounts for non-linear relationships, which may remain undetected using correlation
1029 analysis. A $\text{VIF} > 8$ indicates variance-inflated variables (Zuur *et al.*, 2009). If more than one variable
1030 exhibit high VIFs, we exclude them stepwise, starting with the variable that has the highest VIF.

1031
1032 The second step consisted in the application of the exploratory analysis (Random Forest) which
1033 helped to decide which variables to include in the model of each response variable, bearing in mind,
1034 however that those known to be strongly related to biology should be preferred (Feld *et al.*, 2016).
1035 Random Forest (RF) analysis is a flexible, non-parametric regression tool belonging to the family of
1036 Classification and Regression Tree analyses (CART). RF can handle a large number of predictors even
1037 in small datasets with a low number of observations. RF is suited to analyse non-linear relationships
1038 and complex interactions (Breiman, 2001) and can handle missing values. For this study, we chose to
1039 use `rfsrc()` function in the ForestSRC package (r software), because of its capability to rank, identify
1040 and visualise predictors interactions.

1041 This step aims to quantify and test the significance of single and combined effects of the explanatory
1042 variable candidates identified by RF analysis. To determine if these candidates have significant
1043 additive or interactive effects and to compare the effects, we need to generate standardised effect
1044 sizes (SES), see (Grueber *et al.*, 2011).

1045
1046 The third step consists in modelling. Here, we used multi-model inference to select the final model
1047 for testing multiple-predictors effects for each response variable. In multiple-predictors modelling,
1048 the primary goal is to determine the importance and significance of various additive and combined
1049 (interactive) explanatory variables. Multi-model inference is a more objective method of model
1050 selection providing consensus models and variable importance estimates across a set of “top
1051 models”, instead of focussing on a single model (Grueber *et al.*, 2011).

1052 The R package MuMIn (Barton, 2019) offers a useful set of functions to perform the steps required
1053 from model ranking to averaging. First, a global GLM (full) model including all the variables of interest
1054 is run. Second, the function `dredge()` automatically runs all models possible with different
1055 combinations of predictors variables based on a minimum and maximum number of predictors (1 for
1056 each 10 data; predictors selection based on RF analysis and coherent with the objectives of the
1057 study). Third, we select a set of best models that potentially best describe multiple-predictors
1058 patterns using the function `get.models()`. The argument “subset” allowed restricting the output
1059 based on thresholds of cumulative $\text{AIC} \leq 0.90$, in order to obtain the best model in the set. Thus, we
1060 derived an averaged model for each response variable using the function `model.avg()` based on AIC
1061 values (Grueber *et al.*, 2011).

1062
1063 The last step consists in validate the final model by checking model assumptions in order to represent
1064 a statistically valid linear model, its residuals need to exhibit normality, homoscedasticity (i.e.

homogeneous variability of residuals along the response gradient), without strong influential observations and any spatial or temporal autocorrelation in the residuals (Zuur et al., 2009).

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Supplementary Information S4: RELATE Mantel-like test

RELATE

Testing matched resemblance matrices (Mantel-like test)

Parameters

Rank correlation method: Spearman

Sample statistic (Rho): 0,102

Significance level of sample statistic: 18,2 % (p = 0.182)

Number of permutations: 999

Number of permuted statistics greater than or equal to Rho: 181

Sample sites		Annual precipitation	
● FL	● >1250	1050-1100	800-850
● SD	● 1200-1250	1000-1050	750-800
● MD	● 1150-1200	950-1000	700-750
● LD	● 1100-1150	900-950	650-700
		850-900	600-650
			550-600
			500-550
			450-500
			400-450
			350-400

Figure S1. Map showing the streambed sampling sites selected in Catalonia (ArcGIS v.10). The colour gradient represents the values of annual precipitation (www.icc.cat) and the sites colour indicates the different sites groups (FL: Flow; SD: Short Dry phase; MD: Medium Dry phase; LD: Long Dry phase).

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Fig.S2 Boxplots describing values of extracellular enzyme activities (GLU, XYL, PHE), community respiration (RESP) and diversity indices (Shannon and Richness index) in the four hydrological groups considered in the study (FL, SD, MD and LD sites).

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1119 **Fig.S3** Variance partitioning results obtained for the averaged models created for each functional
1120 (GLU, β -glucosidase; XIL, β -xilosidase; PHE, phenol-oxidase; RESP, community respiration) and
1121 structural (DIV, diversity index; RICH, richness index; BAC, bacterial density) microbial community
1122 descriptor. The hydrological and environmental factors considered in the models are listed in the
1123 legend: forested land-use (FORE); riparian vegetation cover (RIP_VEG); organic matter content
1124 (ORG_MAT); agricultural land-use (AGRI); total dry phase duration over the 8-months (T_DRY);
1125 duration of the last rewetting phase (REW); sediment water content (WAT_CON); total dry-phase and
1126 rewetting interaction (T_DRY*REW).

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1136 **Fig.S4** Bacteria phyla and classes composition. Values are expressed as percentage of relative
1137 abundance (%) and separated in the hydrology groups: FL, flow sites; SD, short dry-phase sites; MD,
1138 medium dry-phase sites; LD, long dry-phase sites.

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