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## Multi-model assessment of hydrological and environmental impacts on streambed microbes in Mediterranean catchments

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► **To cite this version:**

Giulia Gionchetta, Joan Artigas, Rebeca Arias-real, Francesc Oliva, Anna Maria Romaní. Multi-model assessment of hydrological and environmental impacts on streambed microbes in Mediterranean catchments. *Environmental Microbiology*, 2020, 22 (6), pp.2213-2229. 10.1111/1462-2920.14990 . hal-02998717

**HAL Id: hal-02998717**

**<https://hal.science/hal-02998717>**

Submitted on 11 Dec 2020

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1 In Environmental Microbiology, 2020

2

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

5

6 Gionchetta G, Artigas J, Arias-Real R, Oliva F, Romani AM

7

8 **SUMMARY**

9

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.

27

28 **INTRODUCTION**

29

30 Dry streambed sediments are dynamic ecotones where terrestrial and aquatic ecosystems  
31 interact, their functioning is largely driven by microorganisms hosted in the sediment and  
32 shaped by temporal environmental fluctuations (Datry et al., 2017; Arce et al., 2019).  
33 Currently, dry streambeds are expanding worldwide due to increased flow intermittency.  
34 Reduced annual precipitation, increased water extraction and the global temperature rise are  
35 the major factors intensifying flow intermittency, especially in Mediterranean areas (Cipriani  
36 et al., 2014; Schewe et al., 2014). Most Mediterranean intermittent streams, such as those in  
37 the northeastern Iberian Peninsula, usually cease flowing for between 1 and 2 months each  
38 year in the summer period. It has been reported that, currently, intermittent streams may  
39 exhibit extended dry-phase episodes also coupled with intense and sporadic precipitation  
40 (Duran et al., 2017). The increase of streambed aridity in intermittent streams and the  
41 consecutive reduction of sediment-water content due to extended exposure to the air may  
42 affect the hosted microbiota and compromise microbial metabolism (Rees et al., 2006;  
43 Marxsen et al., 2010; Febria et al., 2015; Gionchetta et al., 2019). Microbial streambed  
44 communities are responsible for key ecosystem processes such as nutrient cycling and  
45 biogeochemical processes, and their metabolic capabilities are fundamental for maintaining  
46 fluvial ecosystem functioning (Datry et al., 2017). Studies report that a long period of water  
47 stress reduces specific microbial activities linked to carbon degradation, probably due to: (i)  
48 the transition of microbes to a dormant stage (Schimel et al., 2007; Jones and Lennon, 2010);  
49 (ii) the allocation of a large fraction of cell metabolic costs for developing survival strategies  
50 to cope with desiccation (such as the production of extracellular polymeric substances) (Lake,  
51 2003; Frossard et al., 2012; Romaní et al., 2017); and/or (iii) reduced labile organic matter  
52 available for microbes during droughts and increased accumulation of recalcitrant compounds  
53 on the streambed surface (Ylla et al., 2010; Freixa et al., 2016). On the other hand, and in a  
54 similar way to arid soil systems, frequent or sporadic precipitations interrupting dry periods  
55 can cause sharp exceptional increases in streambed sediment respiration activity, as a result of  
56 the awakening of dormant microbes (Bérard et al., 2011; Evans et al., 2014; Meisner et al.,  
57 2017; Marcé et al., 2019). Streambed microbial assemblages have been shown to be affected  
58 by drying–rewetting cycles, especially when these include unusually extended dry phases  
59 (Pohlon et al., 2013; Meisner et al., 2018). Their taxonomic composition can shift towards a  
60 community able to cope with more extreme environmental conditions, resulting in a more

61 specialized community able to develop specific life strategies for coping with drought (Jones  
62 and Lennon, 2010; Shade et al., 2012; Evans and Wallenstein, 2014). In addition to  
63 hydrology, streambed microbes in intermittent systems may be further affected by co-  
64 occurring environmental factors related to the characteristics of the catchment acting at  
65 different spatial scales. Physical characteristics, such as the type of streambed substrata (e.g.  
66 cobbles/bedrock, fine/coarse sediment, accumulation of leaf litter), can influence the  
67 microbial response to hydrological intermittency (Duarte et al., 2017; Arce et al., 2019).  
68 Headwaters are usually more heavily affected by natural intermittency than downstream areas,  
69 due to their hydro-morphological features (e.g. pronounced slope, distinct geomorphology  
70 reducing the hyporheic zone); also, these areas have been described as microbial biodiversity  
71 reservoirs, reflecting the high variability of habitats and resources (varying quantity and  
72 quality of organic matter), and/or higher levels of microbial colonization from the adjacent  
73 soils than that observed downstream (Besemer et al., 2013). In headwater sections, a greater  
74 accumulation of leaf litter combined with a reduced percentage of sand and gravel sediment  
75 particles could lessen the loss of organic matter and water content during dry periods, limiting  
76 the reduction of microbial activity (Freixa et al., 2016). Other studies have reported that leaf  
77 packs buried in the sediment maintain high humidity under extreme dry conditions, becoming  
78 temporal shelters for microbial survival (Gionchetta et al., 2019). Similarly, the presence of  
79 riparian vegetation, able to curtail water loss from sediments under dry conditions, has been  
80 considered a hydrological refuge for microbiota inhabiting the streambed (McLaughlin et al.,  
81 2017). At a larger scale, catchment land uses can also influence the stream and streambed  
82 biota and microbiota responses, especially through factors relating to the use of the  
83 surrounding soils (e.g. agriculture or industrial activities), or a reduction in the natural riparian  
84 vegetation (Allan, 2004; Steward et al., 2012, 2018; Bruno et al., 2016). To date, most studies  
85 related to the consequences of intermittency on streambed microbes have been mainly focused  
86 on the effects provoked by wet (and rewetting) periods (Timoner et al., 2014). Less attention  
87 has been paid to the drought period, with only a few field studies focused on a single or  
88 limited number of stream ecosystems (Timoner et al., 2012; Zeglin, 2015; Freixa et al., 2016).  
89 However, to better understand streambed microbial responses to intermittency, field studies  
90 embracing the multiple pressures (including the hydrological history and other environmental  
91 factors) are needed, as shown by recent studies demonstrating the interactive and joint effect

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

116

117

## 118 **RESULTS**

119

### 120 **Site classification and catchment characteristics**

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

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

145

### 146 **Influence of hydrological history on streambed microbial functions**

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

154 S3, Table 2). Specifically, the total duration of the dry phase explained the largest variance in  
155 the case of PHE activity (PHE, VAR. PAR. T\_DRY = 13%, Fig. S3), while the interaction  
156 between the total dry duration (T\_DRY) and the length of the last wet period (REW)  
157 influenced the variation in community respiration (RESP, VAR. PAR. T\_DRY\*REW = 13%,  
158 Fig. S3). In contrast, the organic matter content and riparian vegetation cover better explained  
159 the variance of XYL (VAR. PAR. ORG\_MAT = 22%, RIP\_VEG = 25%) while both organic  
160 matter and water content of sediments were relevant for GLU variance explanation (VAR.  
161 PAR. ORG\_MAT = 17%, WAT\_CON = 15%, Fig. S3). Microbial function plots showed the  
162 relationship (significant or not) between each functional variable and the total dry duration as  
163 well as the effect (e.g. additive, synergistic or antagonist) of the duration of the last wet period  
164 (Fig. 2). According to the model plots, despite the high variability seen between the  
165 intermittent sites, the hydrolytic GLU and XYL activities decreased slightly when T\_DRY  
166 increased, and this trend was significant in the case of XYL activity (Fig. 2A and B, Table 2).  
167 In contrast, PHE activity increased significantly with longer dry periods, with mean PHE  
168 activities doubling at LD sites compared with those at SD sites (126.63 13.64 and 251.13  
169 55.19 nmol DIQC gDW<sup>-1</sup> h, at SD and LD sites respectively), while no interaction with  
170 REW was found (Fig. 2C, Fig. S2 and Table 2). With regard to catchment characteristics,  
171 sediment organic matter content was positively related to GLU and XYL hydrolytic activities  
172 (Table 2), while the riparian vegetation cover was negatively related to XYL activity (Table  
173 2). Community respiration presented a more complex response to the hydrological conditions,  
174 with peaks occurring with longer rewetting periods in largely intermittent and extremely dry  
175 sediments (MD and LD sites in Fig. 2D, Table 2).

176

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

185 hydrological parameters, such as T\_DRY, REW and T\_DRYxREW, accounting for 5% of the  
186 diversity, 9% of the richness and 5% of the bacterial density (Fig. S3). Significant positive  
187 relationships were found between both the diversity and richness indices (DIV, RICH) and the  
188 riparian vegetation coverage (RIP\_VEG, Fig. 3, Table 2). Specifically, linear models showed  
189 that abundant riparian vegetation determines a positive and significant increase in both the  
190 microbial diversity and richness indices, particularly at MD sites (Fig. 3A and B; Table 2).  
191 With regard to land-use, agricultural land cover explained the largest part of the bacterial  
192 density variance (BAC, VAR. PAR. AGRI = 33%) and it was also positively and significantly  
193 related to the bacterial density, but negatively related to community diversity (Table 2). The  
194 microbial composition of the sediments changed in response to the duration of the dry phase;  
195 sites with continuous flow had a significantly different community composition to that at  
196 intermittent sites [permutation analysis of variance (PERMANOVA), Fig. 4A]. This result  
197 was confirmed by the relation existing between NMDS1 and PC1 (Fig. 4B). In terms of  
198 taxonomy, the phylum Proteobacteria dominated the streambed communities at all sites,  
199 although its relative abundance decreased at intermittent sites (Fig. S4). Specifically, there  
200 was a significant reduction in the classes Delta-proteobacteria and Epsilon-proteobacteria  
201 between FL and LD sites [analysis of variance (ANOVA)  $p < 0.05$ ; Fig. S4, Fig. 4A].  
202 Similarly, the relative abundance of the phylum Planctomycetes decreased along the dry phase  
203 gradient, with a significant decrease in the relative abundance of the class Planctomycetia at  
204 MD and LD sites compared with FL sites (ANOVA  $p < 0.05$ ; Fig. S4, Fig. 4A). In contrast,  
205 the relative abundance of the phyla Actinobacteria and Bacteroidetes doubled at sites with the  
206 longest dry phase (Fig. S4). More specifically, of the classes Actinobacteria ( $r_s = 0.76$ ),  
207 Thermoleophilia ( $r_s = 0.56$ ) and Sphingobacteria ( $r_s = 0.65$ ) correlated strongly with the MD  
208 and LD communities (Fig. 4A); the relative abundance of Actinobacteria increased  
209 significantly at MD and LD sites (ANOVA,  $p < 0.05$ ; Fig. S4, Fig. 4A), and Sphingobacteria  
210 at LD sites, compared with FL sites (ANOVA,  $p < 0.05$ ; Fig. S4, Fig. 4A). The global  
211 correlation between the community composition and the functions was not significant ( $p =$   
212  $0.182$ ,  $r_s = 0.10$ ), suggesting that these are uncoupled (Supplementary Information S4).

213

214

215 **DISCUSSION**



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

230

### 231 **Drying gradient effects on streambed microbial function**

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

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

276

277 **Drying gradient effects on streambed community composition**

278 The microbial community composition reflects clear differences in the relative abundance of  
279 certain taxa inhabiting the flowing and intermittent sites, suggesting that the presence of no  
280 flow episodes exerts an important effect, irrespective of the degree of intermittency. Microbes  
281 are capable of adapting rapidly to changing environmental conditions through structural and  
282 functional changes (Allison and Martiny, 2008; Wallenstein and Hall, 2012). Consequently,  
283 the stress provoked by a single dry period may determine the changes in intermittent  
284 microbial communities through the rapid osmotic adaptation of cells to cope with alternating  
285 dry and wet periods (Shade et al., 2012; Attermeyer et al., 2013; Meisner et al., 2018). As  
286 expected, in our study, increased dryness tended to modify the microbial communities'  
287 composition with respect to those of the flowing sites, involving higher relative abundances of  
288 drought-tolerant taxa and fewer drought sensitive taxa. This microbial community transition is  
289 characterized by a decrease in Gram-negative bacteria compared with an increase in the  
290 proportion of Gram-positive bacteria, which are more resistant and adapted to live in soil  
291 systems submitted to extreme drought (de Vries and Shade, 2013; Acosta-Martínez et al.,  
292 2014; Naylor, 2017). Furthermore, the microbial composition changes were driven by either  
293 just one or a few members of a microbial phylum, as also observed by Barnard and colleagues  
294 (2013) in soil systems. Specifically, the assemblage shifts in these streambeds included a  
295 significant decrease in the Gram-negative classes of Epsilon-proteobacteria and  
296 Deltaproteobacteria (phylum Proteobacteria) and Planctomycetia (phylum Planctomycetes).  
297 Conversely, we found significant increases in the Gram-positive classes Actinobacteria and  
298 Thermoleophilia (phylum Actinobacteria) as observed in extremely desiccated soils (Bouskill  
299 et al., 2013). The spread of the class Sphingobacteria (phylum Bacteroidetes) in the driest  
300 intermittent communities could be due to rewetting effects (last rewetting event prior to  
301 sampling). Sharp increases in the relative abundance of the class of Sphingobacteria have  
302 often been associated with abrupt wet events in arid and desert soil communities (Aslam et al.,  
303 2016; Meisner et al., 2018). In contrast to community composition, richness and diversity  
304 were not significantly modified by hydrological history, suggesting that there was taxa-  
305 dominance alternation or substitution without increased dryness significantly affecting the  
306 overall diversity. This suggests that desiccation may also play a role in decreasing diversity  
307 and richness with a concomitant decrease in riparian vegetation cover.

308

### 309 **Relevance of environmental catchment characteristics**

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

330

### 331 **CONCLUDING REMARKS**

332

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

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

356

## 357 **EXPERIMENTAL PROCEDURES**

358

### 359 **Study area, site selection and sampling strategy**

360 A total of 37 upstream sites were selected and surveyed in streams situated in the northeastern  
361 Iberian Peninsula (Catalonia). These sites belong to nine watersheds corresponding to the  
362 main rivers in that area, flowing into the Mediterranean Sea, i.e. the Muga, Fluvià, Ter,  
363 Tordera, Llobregat, Francolí, Foix, Besós and Ebro rivers (Fig. S1). The sampling sites  
364 include lowland and midmountain watercourses (altitude 80% totally shaded, considered  
365 length 100 m) and riparian vegetation cover (e.g. the presence of trees, shrubs or small bushes  
366 was considered as a percentage, from 75%, for the first 10 m width of riparian vegetation)  
367 were measured in situ following a fieldassessment procedure. Once at the laboratory,  
368 sediment subsamples of about 1 ml volume (1 cm<sup>2</sup> projected surface area) were created and  
369 collected from each sediment corer, using an uncapped syringe, for EEA, respiration, bacterial  
370 community composition and density analyses. Community respiration and EEA were

371 measured within 24 h of sampling. Bacterial density samples were fixed with 10 ml of  
372 filtersterilized (0.2  $\mu\text{m}$  pore size nylon filters Whatman, Kent, UK) synthetic water (13 mg  
373  $\text{L}^{-1}$   $\text{Na}_2\text{SO}_4$ , 16.1 mg  $\text{L}^{-1}$   $\text{Na}_2\text{SiO}_3$ , 29.4 mg  $\text{L}^{-1}$   $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.6 mg  $\text{L}^{-1}$   $\text{KCl}$ , 3 mg  
374  $\text{L}^{-1}$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 26.5 mg  $\text{L}^{-1}$   $\text{Na}_2\text{CO}_3$ , 0.6 mg  $\text{L}^{-1}$   $\text{NH}_4\text{H}_2\text{PO}_4$  and 7.3 mg  $\text{L}^{-1}$   $(\text{NH}_4)$   
375  $(\text{NO}_3)$  in MQwater) and formaldehyde (2% final concentration), and then stored at room  
376 temperature. For the microbial diversity analysis of the 16S rRNA gene, the sediments were  
377 stored at  $-20\text{C}$ . The remaining sediment was used to determine organic matter and water  
378 content.

379

### 380 **Hydrological history and catchment characteristics of the study sites**

381 One year prior to the sampling, temperature and pressure transducers (Solinst Levellogger  
382 Gold Model 3001 and Solinst Barologger Gold Model 3001, Solinst, Georgetown, ON,  
383 Canada), and temperature data loggers (ACR SmartButton Logger, MicroDAQ) were installed  
384 at the study sites to characterize their hydrology. All the temperature loggers recorded data  
385 every 60 min. Once the entire dataset for each sampling site had been obtained, the daily  
386 variations in the streambed and air temperature were estimated. To characterize the stream  
387 hydrology in the intermittent stream, we used the daily variation of the streambed temperature  
388 corrected for the barometric pressure and air temperature. This daily variation was determined  
389 as the difference between the maximum and minimum temperature per day and the daily  
390 higher rate of change per hour (Colls et al., 2019) (see Supplementary Information S1, Arias-  
391 Real et al., 2018). In addition, the physical and chemical characteristics of the sites were  
392 recorded (Supplementary Information S2). In total, the hydrological data collected covered an  
393 8 month period (245 days) of hydrology history for each stream site, and the related  
394 parameters calculated from this database were: the total number of dry days in the 8-month  
395 period (T\_DRY); the percentage of dry days in the 8-month period (P\_DRY); the duration  
396 (days) of the last dry event prior to the sampling (L\_DRY); the duration of the last rewetting  
397 period (i.e. the number of consecutive wet days prior to the sampling) (REW) and the number  
398 of dry/rewetting cycles over the 8-month period (FREQ). As well as hydrological descriptors,  
399 in situ data corresponding to sediment organic matter, water content, shade cover and riparian  
400 vegetation were collected, and land use in the catchment area of the sites was determined. The  
401 organic matter content was measured as AFDW (Ash-Free Dry Weight) and 1 ml of sediment

402 sample was dried at 70C for 72 h and then burnt for 4 h at 450C using a muffle furnace (AAF  
403 1100, Carbolite, UK). The results were expressed as grams of AFDW per gram of sediment  
404 dry weight (DW). In order to estimate the sediment saturation, sediment water content was  
405 calculated as the percentage of water loss (% difference) between the fresh and DW (1 ml for  
406 each sample). Major land covers (urban zones, agricultural and forested areas, Corine Land  
407 Cover 2012, <https://www.eea.europa.eu/data-and-maps/data/clc-2012-raster>) were calculated  
408 as percentage considering the adjacent 1 km upstream of the sampling point, within the  
409 catchment area.

410

#### 411 Microbial response variables

412 Bacterial density and community respiration. Bacterial density in sediments was determined  
413 through flow cytometry (FACSCalibur, Becton Dickinson), following a protocol adapted from  
414 Amalfitano and Fazi (2008). Sediment samples were sonicated for 1 min, shaken for 30 s, and  
415 sonicated again for 1 min to extract the biofilm from the sediment grains (Ultrasons, Selecta).  
416 A subsample of the obtained extract (1 ml) was pipetted into a glass vial and 9 ml of  
417 detaching solution (distilled water, NaCl at 0.85% final concentration, Tween 20 at 0.5% final  
418 concentration and sodium pyrophosphate at 0.1 M, final concentration) was added to promote  
419 the separation of the cells. The samples were shaken for 30 min (150 rpm, in the dark and at  
420 room temperature), left for 10 min at 4C, and then sonicated with ice for two 1 min cycles.  
421 Next, the samples were shaken again for 1 min and left for 5 min, to allow the sedimentation  
422 of the larger particles, and, finally, 1 ml of supernatant was transferred into a clean sterile  
423 Eppendorf tube. Once the sediment extracts had been obtained, each sample was purified  
424 through Nycodenz (1 ml), added to the bottom of each Eppendorf tube, and centrifuged (14  
425 000 rpm) for 90 min at 4C. The purified sediment extracts (400 µl) were stained with Syto13  
426 (4 µl Fisher, 5 µM solution) and incubated in the dark for 30 min. The stained samples were  
427 counted using flow cytometry (FACSCalibur, Becton Dickinson). To normalize fluorescence  
428 data, a known concentration of bead solution (10 µl of 10<sup>6</sup> beads ml<sup>-1</sup>, Fisher 1.0 µm) was  
429 added to the samples. The bacterial density (BAC) results were expressed as cells × 10<sup>8</sup> per  
430 gram of sediment DW. Resazurin assay was applied to determine community respiration  
431 (RESP), using a modified version of the technique in Haggerty and colleagues (2010). The  
432 resazurin (7-Hydroxy-3H-phenoxazin3-one-10-oxide sodium salt, RAZ) method consists of

433 using a redox dye (blue in the oxidized state) that indicates the respiratory activity of  
434 microorganisms (it turns pink when reduced). Before applying the resazurin dye, we prepared  
435 the sediment extract for each sample replicate from 1 ml of sediment, placed in a sterile vial  
436 with 5 ml of pyrophosphate solution 50 mM (Manini and Danovaro, 2006). Each sample was  
437 sonicated for 1 min in ice in order to preserve the cell structure (Amalfitano and Fazi, 2008),  
438 shaken for 30 s, and sonicated again for 1 min to extract the biofilm from the sediment grains  
439 (Ultrasons, Selecta). One milliliter subsamples of the extract was diluted in 9 ml of filtered  
440 water (0.2  $\mu\text{m}$  pore size nylon filters Whatman, Kent, UK). Aliquots of each sediment extract  
441 (166  $\mu\text{l}$ ) were placed in a transparent microplate (MicroWell™ Nunc™, ThermoFisher  
442 Scientific) and mixed with ringer solution (28  $\mu\text{l}$ ) and resazurin stock substrate (6  $\mu\text{l}$  of 0.35  
443 mM Sigma-Aldrich, USA). The plate was closed with a lid, then incubated (target temperature  
444 20C), and each cycle started with shaking for 90 s (shaking amplitude 3 mm), with a pause of  
445 10 s before each absorbance reading. The incubation period lasted 24 h and the absorbance  
446 was read every 4 min (603 and 570 nm resazurin and resorufin wavelengths). Subsequently,  
447 we used the standard resorufin (pink compound that indicates the conversion of resazurin to  
448 resorufin) curve and the absorbance values obtained after 18 h of incubation to define the  
449 community respiration as micromoles of resazurin respired per grams of DW  $\text{h}^{-1}$  ( $\mu\text{mol RAZ}$   
450  $\text{gDW}^{-1} \text{h}^{-1}$ ).

451

#### 452 Extracellular enzyme activities

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

460

#### 461 Community composition and diversity

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



464 according to the manufacturer's instructions. The DNA concentration and quality in each  
465 sample were measured spectrophotometrically (Nanodrop2000, Thermo Scientific™,  
466 Waltham, USA) and further assessed with PCR (95C × 5 min; 35 cycles: 95C × 1 min; 55C ×  
467 1 min; 72C × 1 min; 72C × 10 min) and 1% agarose gel electrophoresis. The DNA extracts  
468 were stored at –20 °C until sequencing analysis at the Roy J. Carver Biotechnology Center  
469 (University of Illinois, IL, USA). The diversity of prokaryotes (including bacteria and  
470 archaea) was analysed utilizing the primer pair V4\_515F/V4\_806R61 (Caporaso et al., 2011),  
471 targeting the V4 region of the 16S rRNA gene, using Illumina MiSeq technology. We  
472 analysed both bacteria and archaea species in sediments, but the latter was excluded from the  
473 analysis due to extremely low read values. IM-TORNADO pipeline version 2.0.3.2 (Jeraldo et  
474 al., 2014) was used to generate the operational taxonomic unit (OTU) table and assign  
475 taxonomy to the corresponding OTUs ( $\geq 97\%$  sequence similarity). Bacterial taxonomy was  
476 assigned through the Ribosomal Database Project (RDP version 10, Cole et al., 2014).  
477 Diversity analyses were performed using the IMTORNADO generated output BIOM table.  
478 The range of sequencing depth was between 9668 and 127 452 of DNA for total prokaryotes.  
479 OTU tables were generated from the BIOM table using the phyloseq package for analysing  
480 microbiome data, by R software (R version 3.4.1). Rarefaction curves were generated  
481 (rarefy\_even\_depth function, phyloseq package) and the rarefied OTU table was set to the  
482 smallest count of bacterial reads (9211 reads/sample) in order to minimize bias due to  
483 different numbers of total sequences present in the samples. Two alpha-diversity metrics were  
484 calculated from the OTU table, i.e. the Shannon–Wiener index (DIV) and the Chao1 richness  
485 index (RICH), using the phyloseq package by R software (R version 3.4.1). The raw sequence  
486 dataset was deposited in the NCBI Sequence Read Archive database under Accession No.  
487 PRJNA557375.

488

489

#### 490 Data analyses

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

495 wet days prior to the sampling, REW; frequency of dry/rewetting cycles in the 8-month  
496 period, FREQ; sediment water content, WAT\_CON; organic matter content, ORG\_MAT;  
497 riparian vegetation cover, RIP\_VEG; shade cover, SHAD; forested land use, FORE;  
498 agricultural land use, AGRI; urban land use, URBA). Although the hydrological history was  
499 mainly studied as a continuous factor, in order to allow some general analyses, the streambed  
500 sites were classified according to the duration of their dry phase in a similar way to the  
501 proposal of Costigan et al., (2017), and four classes were defined for our study sites: flowing  
502 sites (FL, continuous flow); short-dry phase sites (SD, from 1% to 15% dry days); medium-  
503 dry phase sites (MD, 15%–85% dry days over the 8-month period); and longdry phase sites  
504 (LD, average 85%–100% dry days) (Fig. S1, Table 1). The differences between the  
505 hydrological categories (FL, SD, MD and LD sites) were analysed for EEA, community  
506 respiration, bacterial density and microbial diversity and richness indices, using one-way  
507 ANOVA. To analyse the response of microbial functions (extracellular enzymes and  
508 respiration), and diversity and bacterial density, to the hydrological and catchment  
509 characteristics, we followed the multiple-predictor analytical procedure using survey data (see  
510 Feld et al., 2016). First, we ran exploratory analyses to reduce skewness when necessary:  
511 GLU ( $\beta$ -glucosidase activity) and XYL ( $\beta$ -xylosidase activity) were square-root transformed;  
512 PHE (PHE activity), RESP (community respiration), BAC (bacterial density), DIV (Shannon  
513 diversity index), RICH (Chao1 richness index) and RIP (riparian vegetation) were log-  
514 transformed). Consequently, we standardized and checked the collinearity between the  
515 variables (Feld et al., 2016). Second, using Random Forest (RF) analysis (package ForestSRC,  
516 R software) (Ishwaran et al., 2014) we ranked, identified and visualized all the predictor  
517 interactions for each response variable: hydrological history parameters (T\_DRY, L\_DRY,  
518 REW, FREQ); sediment water and organic matter (WAT\_CON, ORG\_MAT); shade cover  
519 (SHAD); riparian vegetation (RIP\_VEG); and agricultural (AGRI), forested (FORE), and  
520 urban (URBA) land use. The number of trees in the RF was 3000 and the variables used in  
521 each split was set to three (Feld et al., 2016). Afterwards, we applied generalized linear  
522 models to model the response of each variable to single and combined predictors (Feld et al.,  
523 2016; Grueber et al., 2011; Barton, 2019). For each model, we used a maximum of four  
524 predictors, selected considering the RFs (Feld et al., 2016), including two hydrological  
525 predictor terms (T\_DRY and REW), and at least two environmental predictors for each

526 response variable. Subsequently, the multi-model averaging approach (MuMIn R package)  
527 was applied. This procedure generated all possible models and ranked them based on model  
528 weight  $< 0.05$  for all tests. Details on the analytical procedure are presented in Supplementary  
529 Information S3. The differences in microbial community composition between the study sites  
530 were analysed using non-metric multidimensional scaling (NMDS) ordination, based on  
531 Bray–Curtis dissimilarity performed at the OTU taxonomic level. The OTU relative  
532 abundance matrix used for the ordination analyses was previously fourth root transformed. A  
533 PERMANOVA (9999 permutations) was applied to test the significant differences in the  
534 community composition between the four hydrological history categories (PRIMER 6 version  
535 6.1.11 and PERMANOVA+ version 1.0.1). In order to identify the taxonomic classes  
536 significantly related to the OTU distribution, specific vector fitting analysis was applied to the  
537 NMDS (Legendre and Legendre, 1998). A further one-way ANOVA was applied to determine  
538 significant differences between the relative abundances of the taxonomic classes inhabiting  
539 the different hydrological history categories (FL, SD, MD and LD sites). Finally, a Mantel test  
540 was performed between community composition and the functional matrices to assess whether  
541 they responded similarly to flow intermittency (Bray–Curtis dissimilarity for the  
542 compositional matrix and Euclidian distance for the functional data, and then RELATE  
543 analysis with Spearman rank correlation in Supplementary Information S4, PRIMER 6  
544 version 6.1.11 and PERMANOVA+ version 1.0.1).

545

## 546 **ACKNOWLEDGEMENTS**

547

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

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

561

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## 781 **FIGURE LEGENDS**

782

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

787

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

795

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

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

805 abundance) at the study sites (coloured according to hydrological category, legend). The  
806 arrows indicate correlations with taxonomic classes (Spearman coefficient  $r_s > 0.6$ ). Taxa in  
807 bold portray significant differences between FL and MD/LD sites (ANOVA  $p < 0.05$ ). The  
808 PERMANOVA test of the NMDS ordination analysis and subsequent pairwise comparisons  
809 indicated significant differences between hydrological categories ( $p = 0.003$ ) where the flow  
810 site communities were significantly different to the three other intermittent bacterial  
811 communities ( $p = 0.051$ ,  $p = 0.001$ ,  $p = 0.001$  for the FL-SD, FL-MD, and FL-LD  
812 comparisons respectively). There were no significant differences found between the three  
813 intermittent categories ( $p > 0.1$  for all comparisons). B. Relationship between NMDS 1  
814 (community composition) and PC1 (hydrological sites distribution). The adjusted regression  
815 coefficient is 0.54. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

816

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

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

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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).

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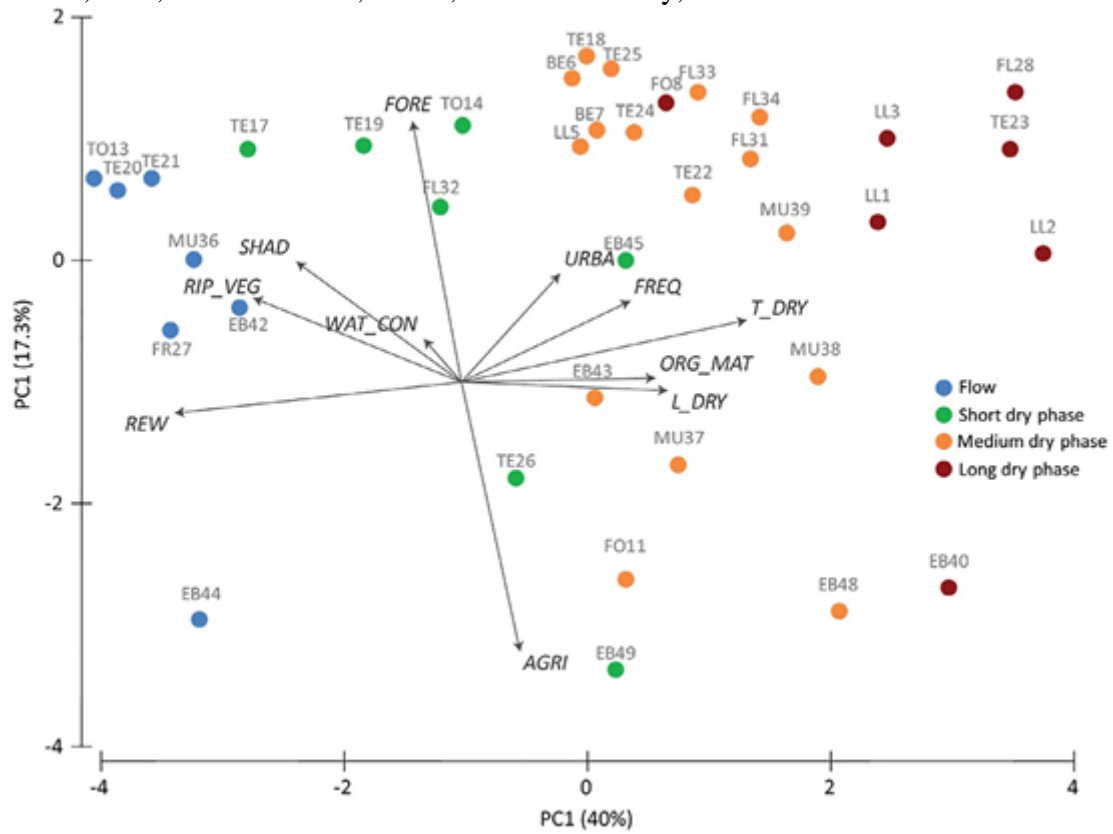
Table 2. Results of the linear models created for each microbial functional and structural response variable.

	SES	SE	p value	mod.rel.imp.	r <sup>2</sup> (%)
<b>GLU</b>					
ORG_MAT	0.633	0.225	<b>0.007**</b>	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
<b>XYL</b>					
RIP_VEG	-0.609	0.177	<b>0.001***</b>	1.00	0.46
ORG_MAT	0.687	0.181	<b>0.001***</b>	1.00	0.46
T_DRY	-0.496	0.209	<b>0.022 *</b>	1.00	0.46
REW	0.042	0.234	0.861	0.27	0.46
T_DRY*REW	na	na	na	na	0.46
<b>PHE</b>					
WAT_CON	0.951	0.585	0.117	0.60	0.21
T_DRY	1.621	0.769	<b>0.041*</b>	1.00	0.21
REW	0.813	0.882	0.375	0.37	0.21
T_DRY*REW	na	na	na	na	0.21
<b>RESP</b>					
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	<b>0.075.</b>	0.27	0.17
<b>DIV</b>					
AGRI	-0.227	0.082	<b>0.004**</b>	1.00	0.53
RIP_VEG	0.285	0.091	<b>0.001***</b>	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	<b>0.014*</b>	1.00	0.53
T_DRY*REW	0.109	0.428	0.809	0.28	0.53
<b>RICH</b>					
FORE	1.731	2.804	0.556	0.23	0.38
RIP_VEG	355.15	97.92	<b>0.001***</b>	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
<b>BAC</b>					
AGRI	0.791	0.176	<b>0.001***</b>	1.00	0.49
RIP_VEG	-0.439	0.200	<b>0.034*</b>	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

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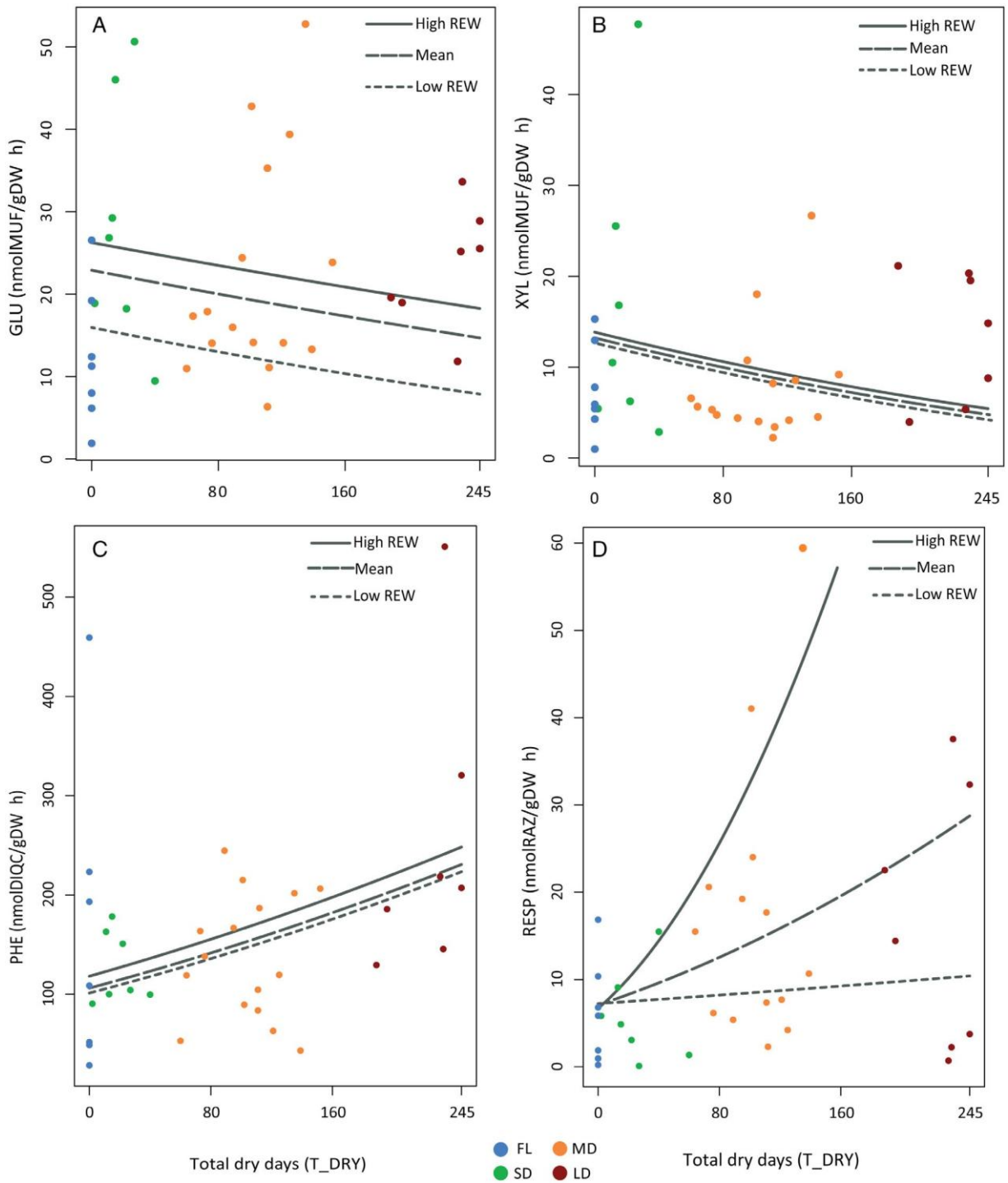
893 These models examined the effect of total dry days (T\_DRY), the rewetting duration prior to  
894 the sampling (REW), their interaction (T\_DRY\*REW) and at least two environmental  
895 predictors. The predictor set for each model was selected considering the results of the  
896 Random Forest analyses. Predictors and interaction standardized effect size (SES), standard  
897 error (SE) and significance (p value) for each model are shown (significant values are  
898 indicated in bold and by: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1). The na interaction values  
899 indicate unavailable results that were automatically excluded from the model as their  
900 importance was too low. The relative predictor importance of the model (mod.rel.imp.) and  
901 the goodness of-fit of the model (r<sup>2</sup>) are also specified for each predictor. The microbial  
902 functional and structural variables considered for modelling were: β-glucosidase, GLU; β-  
903 xylosidase, XYL; phenol-oxidase, PHE; community respiration, RESP; Shannon diversity

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



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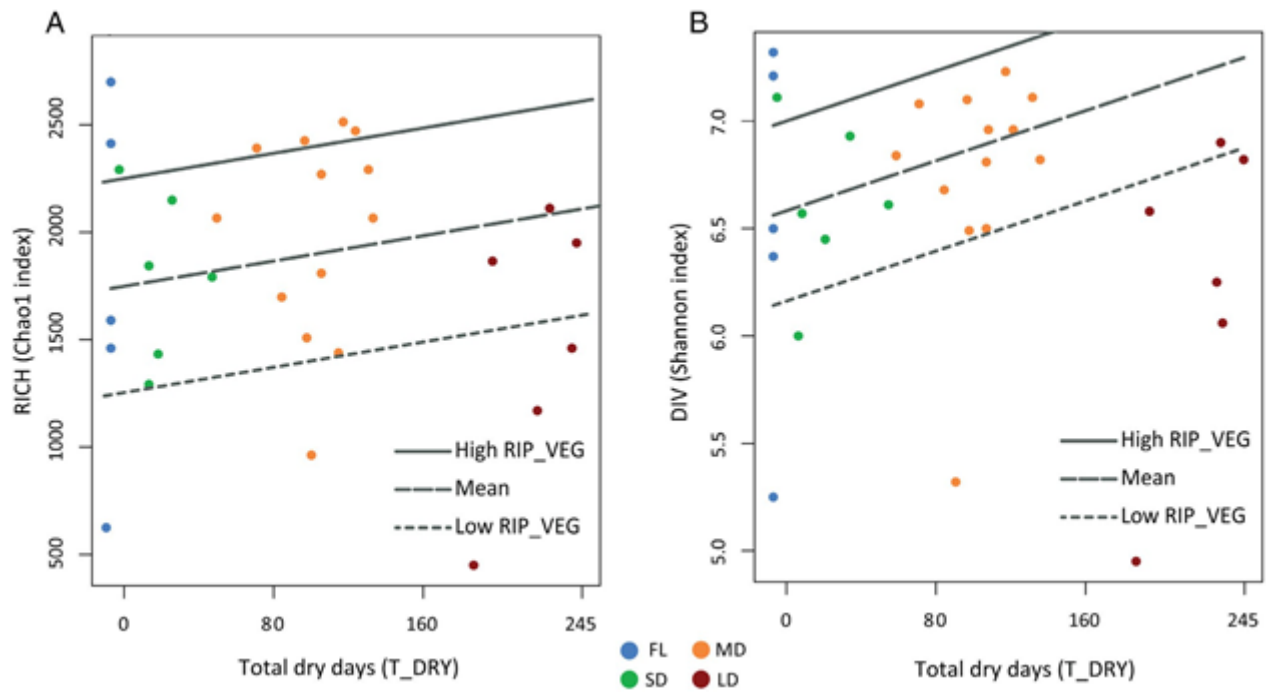
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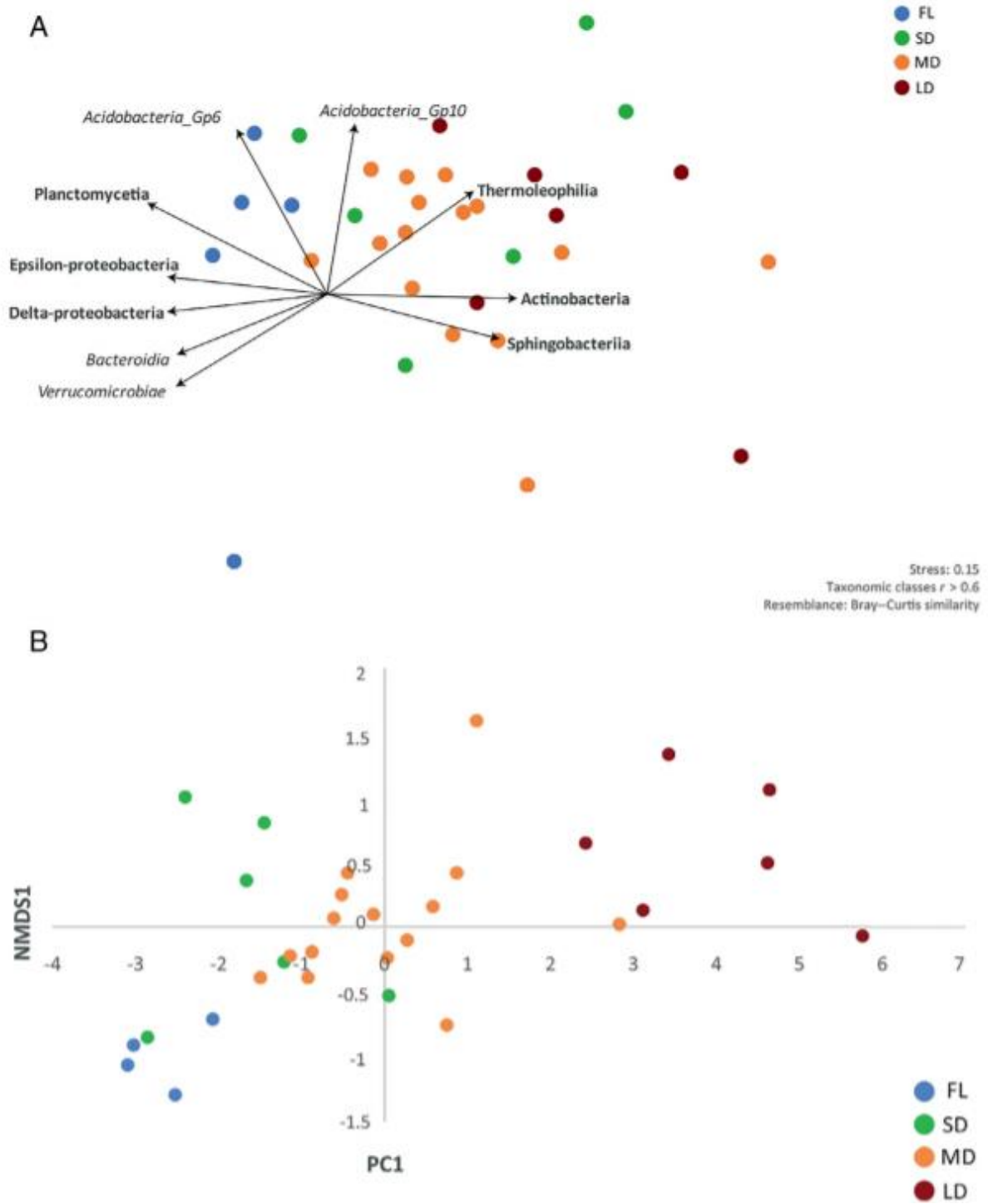
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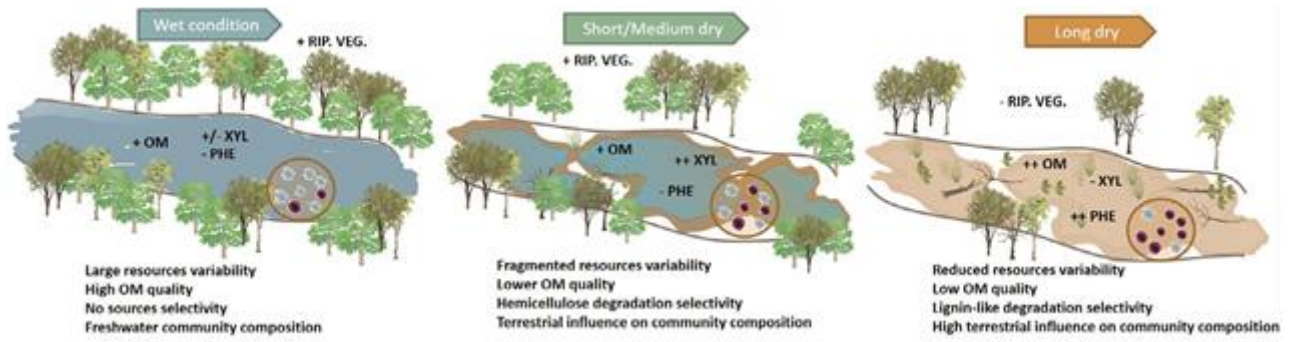


Fig 5

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964 **Supplementary information:**

965 **Table of Contents:**

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969 present in most of the sites), Page 3
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- 976

## 977 **Supplementary information S1: Detailed hydrological measurements**

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

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

999 A moving average of the DA and RTC ratios was calculated, comprising a total of 5 days to overcome  
1000 potential erroneous interpretations of the stream's flow or no-flow state. This allowed dampening  
1001 extreme values and achieving the best fit to water level data. The output value was named  
1002 hydrological status (HS). Starting from the HS serial datasets we calculated all the hydrological  
1003 parameters for each streambed.

1004 For more specific information about the hydrological characterization and to visualize the DA, RTC  
1005 and HS equations we referred to Colls et al. (2019) and Arias-Real et al., (2018), being part of the  
1006 same multi-sites project.

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## 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 1°53'33.00"E	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
	LL5	41°41'46.46"N 1°59'1.67"E	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
Besos	BE6	41°46'1.10"N 2°16'11.98"E	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 2°17'29.77"E	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
Foix	FO8	41°25'7.63"N 1°30'26.44"E	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	41°23'52.63"N 1°35'37.29"E	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
Tordera	TO13	41°51'55.61"N 2°35'35.62"E	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
	TO14	41°41'40.88"N 2°29'0.21"E	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
Ter	TE17	42°5'20.88"N 2°35'17.97"E	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	42°7'17.10"N 2°13'11.86"E	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	42°2'52.10"N 2°24'38.68"E	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	42°4'18.76"N 2°32'28.68"E	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	42°4'39.68"N 2°20'19.50"E	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	42°10'25.26"N 2°10'21.70"E	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
	TE24	41°59'14.24"N 2°50'15.19"E	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
	TE25	42°6'35.03"N 2°29'20.27"E	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
	TE26	41°55'15.45"N 2°42'41.04"E	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
Francoi	FR27	41°18'38.37"N 1°5'2.51"E	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
Fluvia	FL32	42°7'37.99"N 2°38'26.65"E	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
	FL33	42°7'28.45"N 2°26'29.72"E	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
	FL34	42°6'51.11"N 2°26'53.48"E	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
Muga	MU36	42°19'2.62"N 2°42'13.25"E	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
	MU37	42°23'15.61"N 3°3'6.24"E	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
	MU38	42°23'6.91"N 3°1'59.28"E	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
Ebro	EB40	40°59'51.89"N 0°8'5.54"E	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
	EB42	41°15'24.80"N 0°56'34.58"E	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
	EB43	41°12'14.84"N 0°14'57.05"E	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
	EB44	40°52'11.42"N 0°9'36.60"E	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
	EB45	40°58'48.19"N 0°24'24.60"E	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
	EB48	41°0'5.81"N 0°23'4.08"E	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
	EB49	41°0'35.87"N 0°23'31.90"E	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

1019 Component analysed: Dissolved organic carbon, DOC; Total nitrogen, TN; Soluble reactive phosphorous, SRP; Ammonium,  
1020  $\text{NH}_4^+$ ; Calcium,  $\text{Ca}^+$ ; Potassium,  $\text{K}^+$ ; Silicon,  $\text{Si}^+$ ; Sodium,  $\text{Na}^+$ ; Magnesium,  $\text{Mg}^{2+}$ ; Manganese,  $^{2+}$ ; Iron,  $\text{Fe}^{2+}$ ; Chlorine,  $\text{Cl}^-$ ;  
1021 Nitrite,  $\text{NO}_2^-$ ; Nitrate,  $\text{NO}_3^-$ ; Sulphate,  $\text{SO}_4^{2-}$ .

## 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  $\text{VIFs} > 8$  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.

1065 homogeneous variability of residuals along the response gradient), without strong influential  
1066 observations and any spatial or temporal autocorrelation in the residuals (Zuur et al., 2009).

1067

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1084 **Supplementary Information S4: RELATE Mantel-like test**

1085

1086 RELATE

1087 Testing matched resemblance matrices (Mantel-like test)

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1089 *Parameters*

1090 Rank correlation method: Spearman

1091

1092 Sample statistic (Rho): 0,102

1093 Significance level of sample statistic: 18,2 % (p = 0.182)

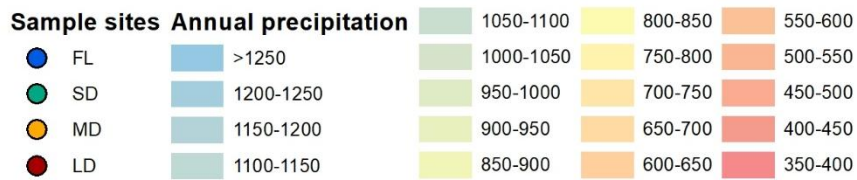
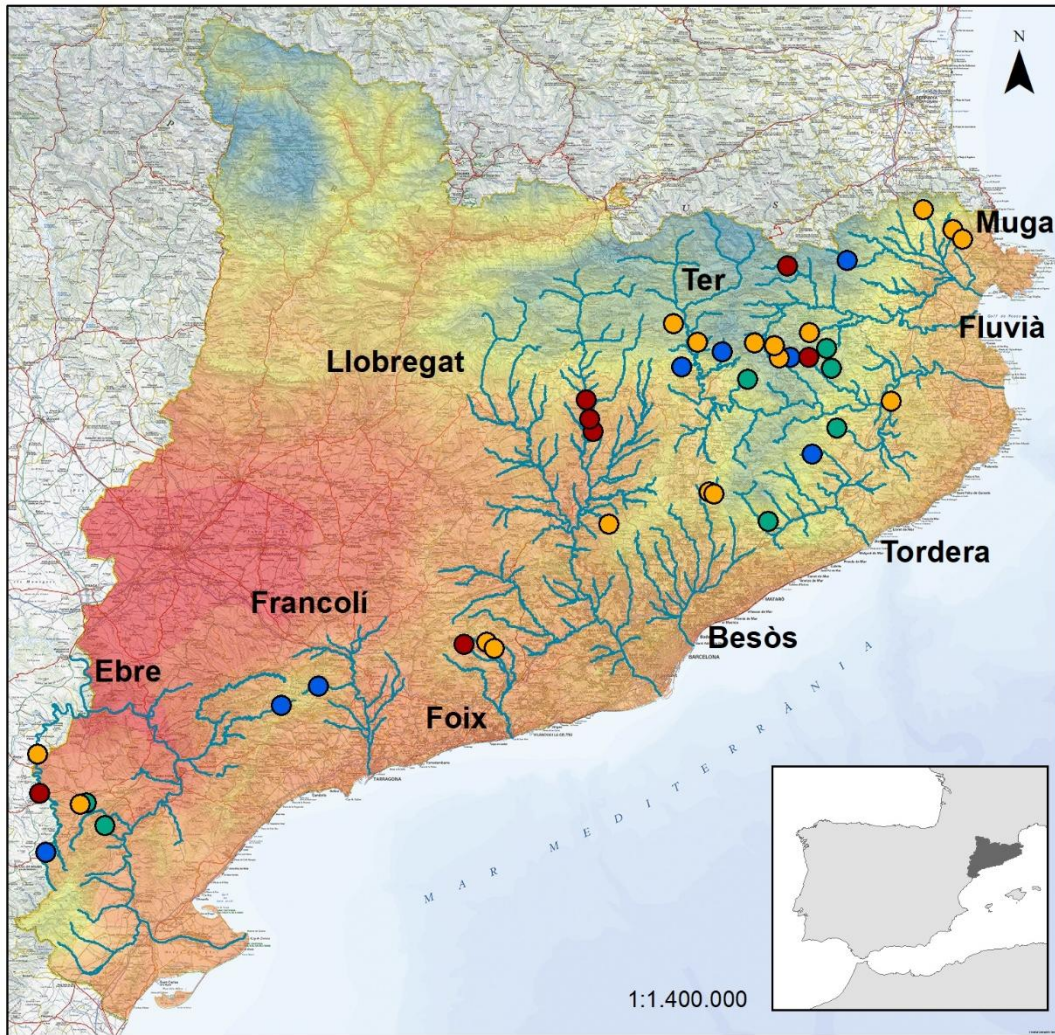
1094 Number of permutations: 999

1095 Number of permuted statistics greater than or equal to Rho: 181

1096

1097





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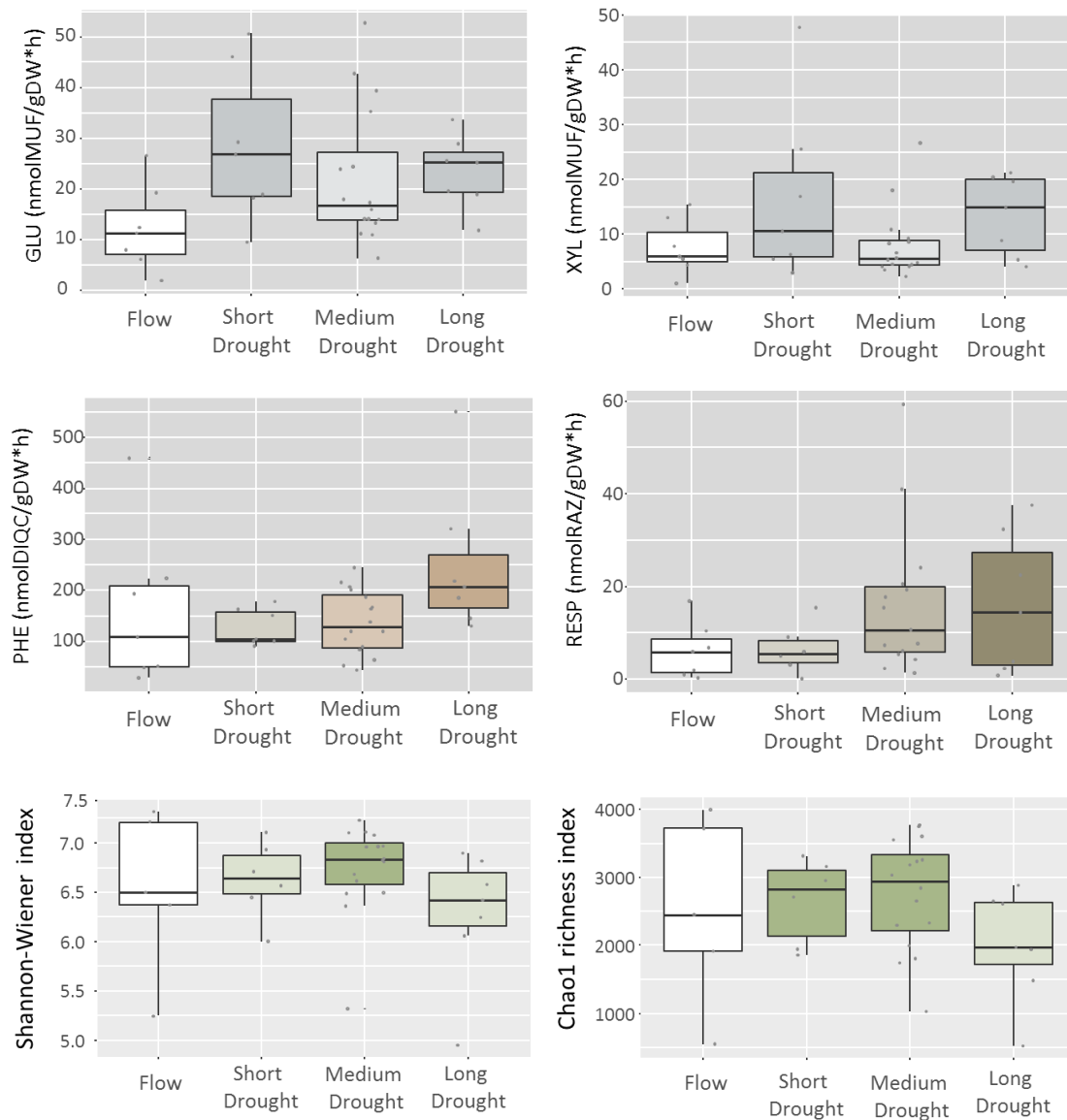
1099 **Figure S1.** Map showing the streambed sampling sites selected in Catalonia (ArcGIS v.10). The colour

1100 gradient represents the values of annual precipitation (www.icc.cat) and the sites colour indicates

1101 the different sites groups (FL: Flow; SD: Short Dry phase; MD: Medium Dry phase; LD: Long Dry

1102 phase).

1103



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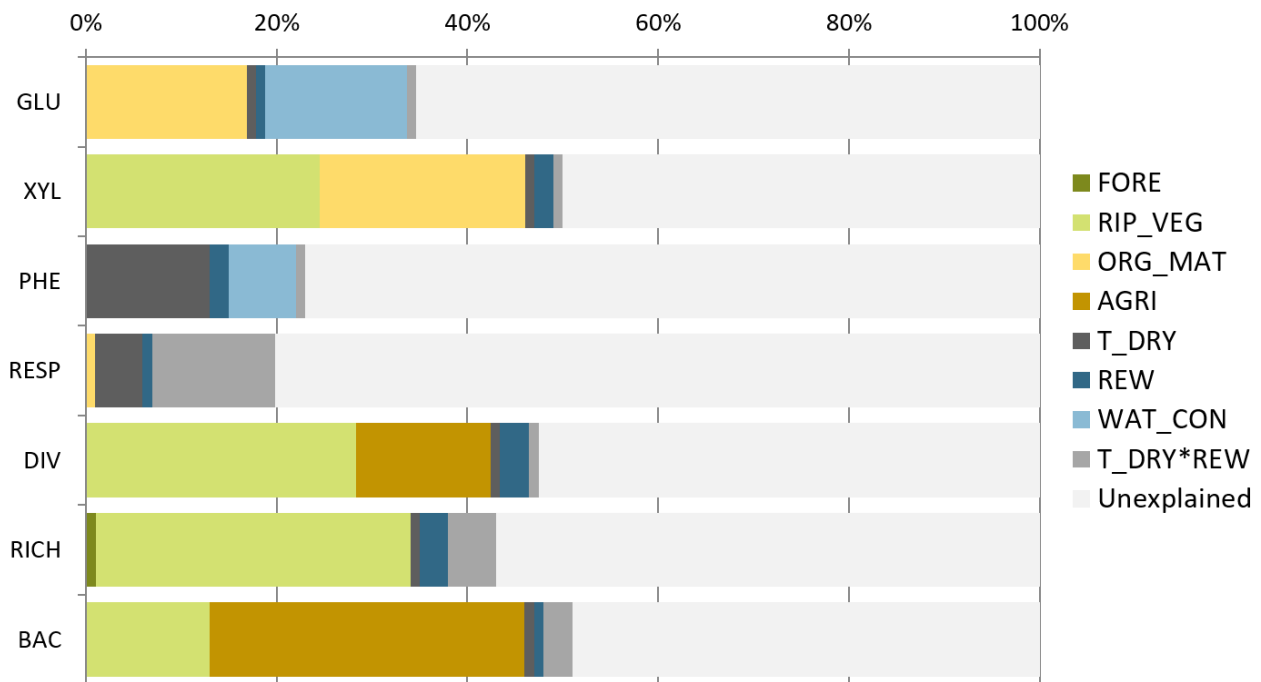
1106 **Fig.S2** Boxplots describing values of extracellular enzyme activities (GLU, XYL, PHE), community  
 1107 respiration (RESP) and diversity indices (Shannon and Richness index) in the four hydrological groups  
 1108 considered in the study (FL, SD, MD and LD sites).

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1119 **Fig.S3** Variance partitioning results obtained for the averaged models created for each functional  
1120 (GLU,  $\beta$ -glucosidase; XYL,  $\beta$ -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).

1127

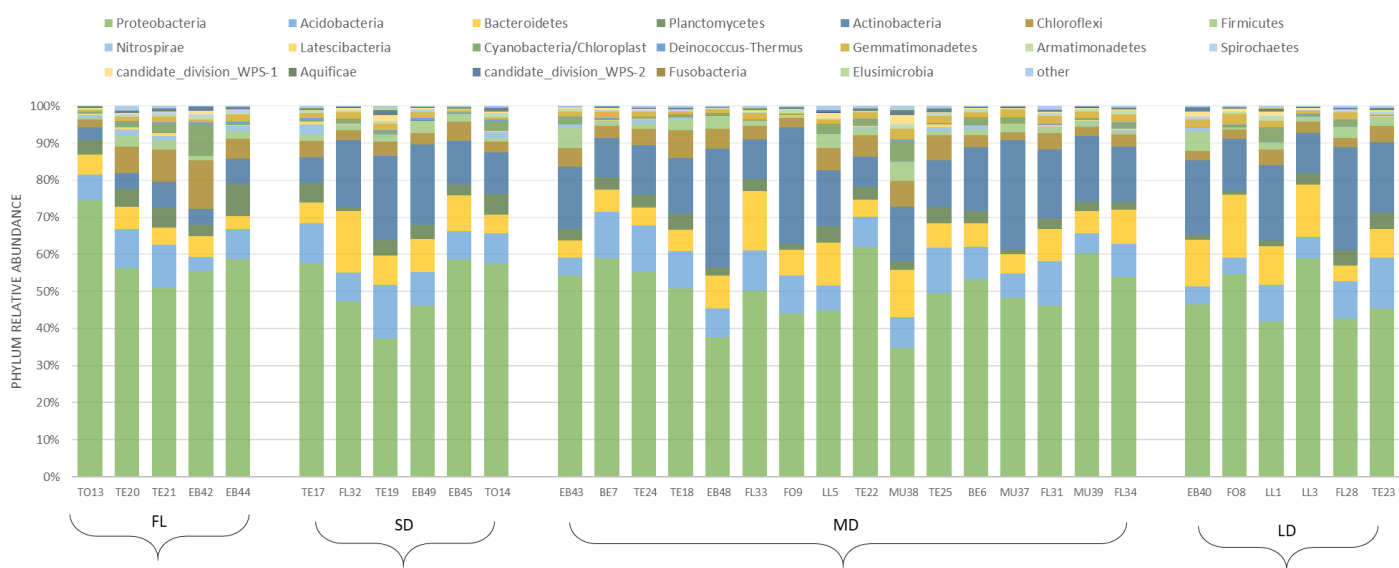
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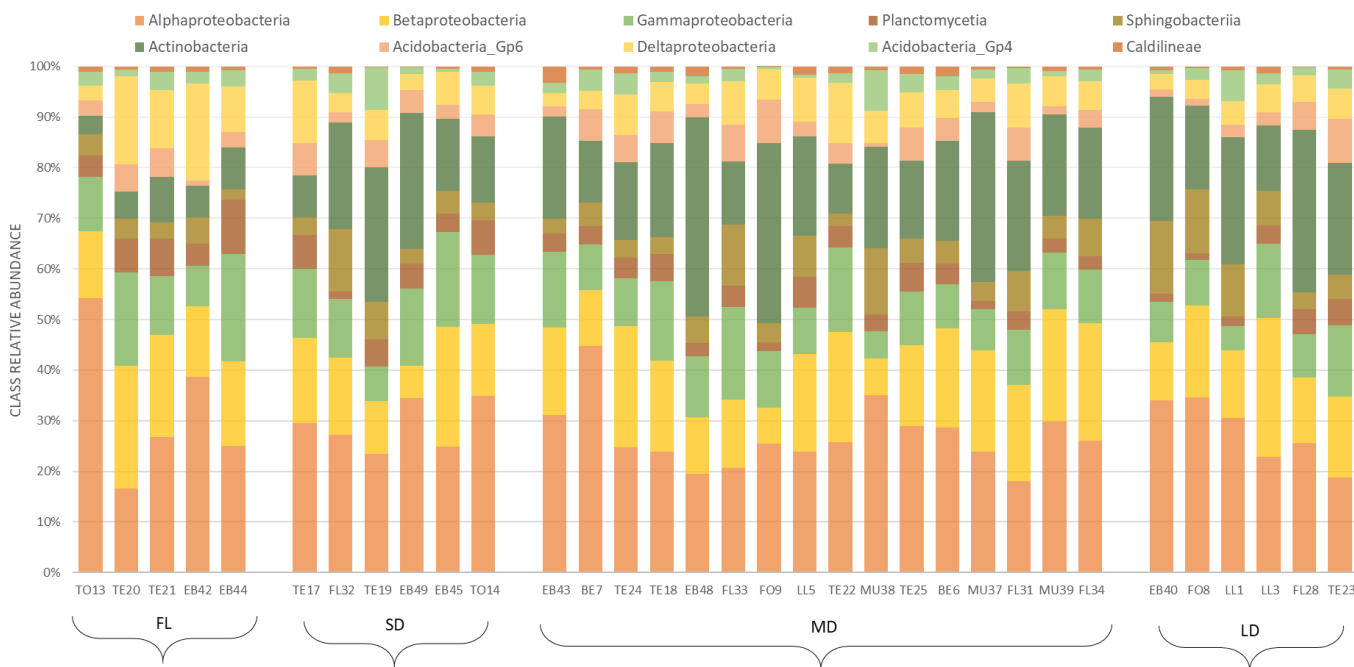
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1136 **Fig.S4** Bacteria phyla and classes composition. Values are expressed as percentage of relative  
1137 abundance (%) and separated in the hydrology groups: FL, flow sites; SD, short dry-phase sites; MD,  
1138 medium dry-phase sites; LD, long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

1139

1140