AT THE EDGE OF AQUATIC SYSTEMS: INTERMITTENT STREAMBED MICROBIAL COMMUNITIES' RESPONSES TO HYDROLOGICAL ALTERATIONS

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Doctoral Thesis

At the edge of aquatic systems: intermittent streambed microbial communities' responses to hydrological alterations

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Dr. Anna M. Romaní Cornet from Universitat de Girona and Dr. Joan Artigas Alejo from Université Clermont Auvergne,

CERTIFY THAT:

That the thesis entitled: “At the edge of aquatic systems: intermittent streambed microbial communities’ responses to hydrological alterations” presented by **Giulia Gionchetta** to obtain a doctoral degree, has been complied under our supervision and meets the requirements to opt for an International Doctorate.

In witness whereof and for such purposes as may arise, the following certification is signed:

Dr. Anna M. Romaní Cornet **Dr. Joan Artigas Alejo**

Girona, 2019

To my dad.

Thanks

And so, it is… I reach the end of my beloved journey of the last four years :)

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List of publications

List of publications derived from this doctoral thesis, one of them are published in international journals.

Gionchetta G., Oliva F, Menéndez M, Lopez Laseras P, Romaní AM. Key role of streambed moisture and flash storms for microbial resistance and resilience to long- term drought. Freshw Biol. 2019;64:306–322. https://doi.org/10.1111/fwb.13218

Gionchetta G., Romaní A.M., Oliva F., Artigas J. Distinct responses from bacterial, archaeal and fungal streambed communities to severe hydrological disturbances. *Scientific Reports. In press.*

Gionchetta G., Oliva F., Romaní A.M., Bañeras L. Total, active and functional streambed microbial diversity: comparing prokaryote and eukaryote responses to hydrological constrains. *Manuscript under review.*

Gionchetta G., Artigas J., Arias-Real R., Oliva F., Romaní A.M. Multi-model assessment of hydrological history impact on microbial structural and functional responses in Mediterranean catchments. *Manuscript under review.*

List of abbreviations and acronyms

AFDW, Ash Free Dry Weight

AGRI, Agricultural land-use

AIC, Akaike's Information Criterion

ANOVA, Analysis of Variance

AWCD, Average Well Color Development

BAC, Bacterial density

BEST, Biota and/or Environment matching

BIC, Schwarz's Bayesian Criterion

cDNA, Complementary DNA

Chl-a, Chlorophyll content

CLPP, Carbon substrate utilization profiles

dbRDA, Distance-based Redundancy Analysis

Dist-LM, Distance-based linear models

DIV, Shannon-Wiener diversity index

DNA, Deoxyribonucleic acid

DO, Dissolved oxygen

DW, Dry weight

EEA, Extracellular Enzyme Activity

EPS, Extracellular polymeric substances

FORE, Forested land-use

FREQ, Frequency of dry/wet cycles

GLM, Generalised lineal model

GLU, ß-glucosidase

L-DOPA, Dihydroxyphenylalanine

L_DRY, Duration (days) of the last dry event

LMM, Linear mixed models

MUF, Methylumbelliferone

NMDS, Non-metric multidimensional scaling

OM or ORG_MAT, Organic matter content

OTU, operational taxonomic unit

PCA, Principal Component Analysis

PCO, Principal Coordinates Analysis

PCR, Polymerase Chain Reaction

P_DRY, Percentage of dry days over the 8-months

PERMANOVA, Permutational multivariate analysis of variance

PHE, Phenol-oxidase

RELATE, Matrix comparison analysis

RESP, Community respiration

REW, Duration (days) of last rewetting period

RICH, Richness index

RIP_VEG, Riparian vegetation cover

RF, Random Forest

RNA, Ribonucleic acid

rRNA, Ribosomal ribonucleic acid

SHAD, Shadow cover

T_DRY, Total dry phase duration over the 8-months

XYL, ß-xylosidase

VAR.PAR., Variance partition,

WAT_CON or WC, Sediment water content

URBA, Urban land-use

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Summary

Hydrological drought is a process of natural desiccation mainly due to large shortage of rainfall events. Reduced precipitations and thus prolonged droughts are spreading worldwide and threaten the integrity of aquatic ecosystems. The reduction of water recharge and the loss of freshwater ecosystems are the most urgent issues of this century, as the world's population grows the demand for water mounts and pressure on finite water resources intensifies. Across Europe the recurrent and intense presence of drought episodes is already a tangible reality affecting stream and river ecosystems.

Mediterranean climate areas, as well as arid and semiarid regions, are particularly prone to prolonged desiccation periods. Most Mediterranean streams have an intermittent flow pattern which may become more variable and with strengthening the duration of their no-flow periods under climate change. Despite intermittent cycles are already characterized by wet-dry-wet phases alternation, the enlargement of the dry period can importantly influence the ecosystem functioning, altering the microbiota inhabiting the streambed sediment as well as the processes they carry out (e.g. nutrients cycling). The streambed surface and the inner hyporheic zones are habitats where microorganisms live in the form of biofilms. These biofilms are responsible for in-stream nutrient cycling and organic matter decomposition. Consequently, the prolonged hydrological depletion and abrupt rewetting episodes can reduce, limit or change microbial community functions, structure and composition, and therefore compromise the overall aquatic ecosystem functioning.

The main objectives of this thesis are to study the responses of the streambed microbial communities (bacteria, archaea, and fungi inhabiting surface and hyporheic sediments and buried leaves) in terms of structure, composition and functions to prolonged dry phase events and to wet episodes, spacing from punctual rains to rewetting events. To meet these objectives, a laboratory experiment (simulating long-term drought, punctual storms and rewetting) in microcosms under controlled conditions and a field experiment were performed to compare microbial responses under hydrological constrains. Specifically, the field study consisted in a snapshot of the streambed microbial structure and function in 37 stream sites with different degree of intermittency that were hydrologically monitored during the 8 months previous to the sampling.

The microbial enzyme capabilities and the biofilm structure observations from the field approach and the laboratory experiment came to complementary results which highlighted relevant responses of streambed microbes to drought. Results from the field experiment

revealed that in sites with permanent flow, streambed microbes generally showed high degradation capacity of simple polysaccharides derived from autochthonous material as well as from cellulose and hemicellulose compounds derived from allochthonous organic matter inputs arriving from the riparian forest. In contrast, sites submitted to no-flow periods and a prolonged desiccation of the streambed enhanced the accumulation and degradation of more recalcitrant compounds such as lignin, which may be due to the accumulation of leaves and wood debris on the dried sediment surface. Similarly, in the laboratory experiment the enzymes linked to the degradation of simple polysaccharides were reduced under long-term drought, mainly in the surface sediments. Also, the laboratory experiment revealed the relevance of the rewetting and storm events for the recovery of surface sediment functioning. Interestingly, the simulated long-term drought showed the stimulation of extracellular polymeric substances (EPS) production in the biofilm, which is a clear microbial-mediated strategy to cope with dryness by retaining a maximum of water in the EPS matrix.

Otherwise, bacterial biomass and the overall diversity of these communities were more resistant to the water stress, with the exception of bacterial cells viability that reported a significant reduction under prolonged desiccation period. Analyses of microbial communities' responses at different streambed microhabitats revealed that the hyporheic sediment and leaf packs were habitats in which the water content was better maintained during the drought and this might reduce un-needed production of extracellular polymeric substances, and allow maintaining the enzymatic activities, bacterial biomass and viability in the biofilm. In terms of bacterial community structure and composition, the field work revealed variations in the microbial diversity mostly related to the environmental features of the catchment (for instance greater agricultural land use and reduced riparian vegetation cover significantly decreased bacterial community diversity). However, similar to that observed in the laboratory experiment, the responses of the microbial community composition to long-term drought revealed that the relative abundance of certain microbial taxa in stream sediments was significantly variable under water stress. The changes observed suggested the terrestrialization bias of the microbial community composition, in transition from intermittently aquatic to more terrestrial ecosystems. Specifically, during the drought period Thermoplasmata class within archaea, and Actinobacteria and Bacilli classes within bacteria increased in response to the water deficit, showing high resistance and a transition towards a soil-like microbial community. Archaea, bacteria and fungi showed distinct sensitivities to hydrological changes, where archaea were the most sensitive followed by bacteria, whereas fungi appeared as the most resistant to these hydrological changes.

Finally, among the molecular and functional tools applied throughout the thesis to describe the diversity of microbes present (DNA-approach), of active microbes (RNA-approach) and of their functions (community level physiological profile CLPP-approach), no redundant information was found. This suggest that these techniques are complementary when analysing the microbial dynamics under hydrological constrains in streams.

Surface sediment is the most threatened streambed habitat to drought since it is subjected to most direct influence of desiccation (e.g. lack of surface water, reduction of interstitial water and high light irradiance) and this greatly affects the inhabiting microbial communities. We observed that streambed microbiota was vulnerable to the hydrological alterations affecting intermittent streams and this can be considered alarming in light of intermittent stream ecosystem functioning and conservation.

Resumen

La sequía hidrológica es un proceso de desecación natural principalmente debido a una gran escasez de lluvias. La precipitación reducida y por tanto la sequía prolongada se está extendiendo por todo el mundo y amenazan los ecosistemas de agua dulce. La reducción de la recarga de agua y la pérdida de los ecosistemas de agua dulce son los asuntos más urgentes de este siglo, debido al aumento de la demanda de agua y a la intensificación de la presión sobre los recursos de agua disponible. En Europa, la presencia recurrente e intensa de eventos de sequía es ya una realidad tangible que afecta a los ecosistemas fluviales, entre ellos ríos y arroyos.

Las zonas de clima Mediterráneo, así como las regiones áridas y semiáridas, son particularmente propensas a largos periodos de sequía. La mayor parte de ríos Mediterráneos tienen un patrón de flujo intermitente que con la influencia del cambio climático puede volverse aún más variable con fases secas más frecuentes y más prolongadas en el tiempo. A pesar de que los ríos intermitentes se caracterizan por la alternancia entre fases secas y húmedas, la prolongación de la duración del periodo seco puede influir de manera importante sobre el funcionamiento del ecosistema, alterando las comunidades microbianas que viven en el sedimento de los ríos así como los procesos que éstas desarrollan a nivel del ecosistema (por ejemplo, los ciclos de nutrientes). El sedimento superficial y las zonas hiporreicas más internas son hábitats de gran diversidad microbiana donde los microorganismos viven en forma de biofilms. Estos biofilms son responsables de procesos como los ciclos de nutrientes y la degradación de materia orgánica. Por lo tanto, una escasez hidrológica prolongada con episodios de precipitación repentinos puede reducir, limitar o cambiar las funciones de la comunidad microbiana, su estructura y composición taxonómica, pudiendo finalmente poner en riesgo el funcionamiento global del ecosistema acuático.

Los principales objetivos de esta tesis son el estudio de las respuestas estructural, funcional y de composición de las comunidades microbianas (bacterias, arqueas y hongos que habitan en el sedimento superficial y hiporreico y también en las hojas enterradas) a la sequía de larga duración y a las fases acúaticas, abarcando desde precipitaciones puntuales hasta muy intensas. Con el fin de alcanzar estos objetivos, fue realizado un experimento de laboratorio (simulando una sequía prolongada, lluvias puntuales y la vuelta de agua al lecho del río) con microcosmos bajo condiciones controladas y un experimento de campo, con el fin de hacer una comparación de las respuestas microbianas bajo restricciones de agua. En concreto, para el estudio de campo 37 puntos de muestreo fueron seleccionados para el estudio de sus

comunidades microbianas y fueron monitoreados desde el punto de vista hidrológico durante los 8 meses anteriores al muestreo, con el fin de conseguir datos hidrológicos y caracterizar los puntos de muestreo.

Las respuestas enzimáticas microbianas y la estructura del biofilm observadas en el estudio de laboratorio y de campo, lograron resultados complementarios manifestando importantes respuestas microbianas a la sequía. Los resultados del experimento de campo indican que las comunidades microbianas de los sitios que tenían siempre agua (ríos permanentes) tenían generalmente una gran capacidad de degradación de polisacáridos simples derivados de la entrada de material autóctono y también de compuestos de celulosa y hemicelulosa derivados de las entradas de materia orgánica alóctona procedente del bosque de ribera. Contrariamente, en los sitios intermitentes y con una fase seca de larga duración, aumentó la acumulación y la degradación de compuestos más recalcitrantes como la lignina, probablemente debido a la acumulación de hojas y de madera sobre el lecho del río seco. De manera similar, los resultados del estudio de laboratorio indican que las enzimas responsables de la degradación de polisacáridos simples se redujeron sobre todo en el sedimento superficial. Además, el experimento de laboratorio resaltó la importancia de los momentos de precipitación y de las tormentas para la recuperación del funcionamiento del sedimento superficial. La sequía de larga duración simulada en el laboratorio resultó en un incremento de la producción de compuestos extracelulares poliméricos (EPS) en el biofilm que es claramente una estrategia de las comunidades microbianas para aumentar la retención de agua y humedad en la matriz de EPS ante la sequedad.

Por otro lado, la biomasa bacteriana y la diversidad total de estas comunidades resultaron ser más resistentes a la escasez de agua, a excepción de la viabilidad celular, que se vio muy afectada y reducida durante el periodo de sequía prolongado. Los análisis de las respuestas de las comunidades microbianas de diferentes microhábitats mostraron que el sedimento hiporreico y las hojas enterradas eran hábitats donde el contenido de agua se mantenía mejor durante la sequía y esto permitía modular la producción de sustancias poliméricas extracelulares, así como regular y mantener las actividades enzimáticas, la biomasa bacteriana y la viabilidad en el biofilm. Desde el punto de vista de la estructura y composición de la comunidad bacteriana, el trabajo de campo indicó que la diversidad estaba afectada por las características ambientales de la cuenca (por ejemplo un aumento de los usos agrícolas del suelo y una menor cobertura de vegetación de ribera produjeron una disminución significativa de la diversidad microbiana).

Sin embargo, como ya ocurrió en el experimento de laboratorio, las respuestas en la composición de la comunidad microbiana a la sequía de larga duración indicaron que la

abundancia relativa de algunas especies presentes en el sedimento se vio muy afectada por la escasez de agua. Los cambios observados sugieren una transición de la comunidad microbiana de acuática a terrestre. En concreto, durante la sequía la abundancia de los grupos taxonómicos Thermoplasmata (archaea) y Actinobacteria y Bacilli (bacteria) aumentó, mostrando una gran resistencia a la ausencia de agua y una transición hacia una comunidad microbiana terrestre. Las arqueas, bacterias y hongos mostraron tener diferentes sensibilidades a los cambios hidrológicos, siendo las arqueas las más sensibles seguidas de las bacterias, mientras que los hongos resultaron ser los más resistentes a los cambios hidrológicos.

Finalmente, las herramientas moleculares y funcionales utilizadas en estos estudios para caracterizar la diversidad microbiana total (ADN), activa (ARN) y funcional (CLPP) no resultaron redundantes. Estas herramientas fueron complementarias cuando se analizaba la dinámica de los microorganismos bajo cambios hidrológicos en los ríos. Entre los hábitats considerados, el sedimento superficial es el más amenazado debido a la falta de agua superficial, la irradiación directa y la perdida rápida de humedad, que afectan de manera importante a las comunidades microbianas.

En general, hemos observado que los microorganismos que viven en el lecho (sedimento) de los ríos son vulnerables a los cambios hidrológicos que afectan a los ríos intermitentes y esto puede resultar peligroso para el funcionamiento del ecosistema y su conservación.

Resum

La sequera hidrològica és un procés de dessecació natural principalment degut a una gran escassetat de pluges. La reducció en les precipitacions i per tant la sequera perllongada s'està estenent a tot el món amenaçant els ecosistemes d'aigua dolça. La reducció de la recàrrega d'aigua i la pèrdua dels ecosistemes d'aigua dolça formen part dels problemes més urgents del nostre segle, a causa de l'augment de la demanda d'aigua i la intensa utilització dels recursos d'aigua disponible. A Europa, la presència recurrent i intensa d'esdeveniments de sequera és una realitat molt important que afecta els sistemes fluvials, incloent especialment rius i rieres de capçalera.

Les zones de clima Mediterrani, així com altres àrees àrides i semiàrides, són propenses a patir sequeres de llarga durada. La major part de rius Mediterranis tenen un flux intermitent que en condicions de canvi climàtic pot esdevenir encara més variable amb fases seques més freqüents i més llargues en el temps. Tot i que els rius intermitents estiguin caracteritzats per una alternança entre fases seques i humides, l'ampliació de la durada del període sec pot influir de manera important en el funcionament de l'ecosistema, alterant les comunitats microbianes que viuen en el sediment dels rius i els processos que desenvolupen a nivell de l'ecosistema (per exemple els cicles de nutrients). El sediment superficial i profund són zones on els microorganismes viuen en forma de biofilm. Els biofilms són responsables de processos com el cicle de nutrients i la degradació de matèria orgànica. Per tant, una escassetat hidrològica perllongada amb episodis de precipitació inesperats pot reduir, limitar o canviar les funcions de la comunitat microbiana, la seva estructura i composició taxonòmica, comprometent així el funcionament global de l'ecosistema aquàtic.

El principal objectiu d'aquesta tesi és l'estudi de les respostes estructurals, funcionals i de composició de les comunitats microbianes (bacteris, arqueus i fongs que colonitzen el sediment superficial i profund i també residus vegetals en procés de descomposició com la fullaraca de la vegetació de ribera que es pot trobar enterrada al sediment) a la sequera de llarga durada i també als moments puntuals d'humitat o rehidratació, com poden ser precipitacions puntuals o molt intenses que determinen la recàrrega de l'ecosistema fluvial. Per tal d'assolir aquests objectius, es va realitzar un experiment de laboratori (simulant una sequera perllongada, pluges puntuals i recuperació de condicions de saturació d'aigua) amb microcosmos sota condicions controlades i, per altra banda, es va portar a terme un mostreig de camp intensiu, per tal de fer una comparació de les respostes microbianes en un rang ampli de condicions hidrològiques. En concret, per a l'estudi de camp, es van seleccionar 37 punts de

mostreig per a l'estudi de les seves comunitats microbianes i van ser monitoritzats des del punt de vista hidrològic durant els 8 mesos previs al mostreig, per tal d'aconseguir dades hidrològiques i caracteritzar els punts en funció del seu grau d'intermitència específic.

Les respostes de les activitats enzimàtiques microbianes i de l'estructura del biofilm observades en l'estudi de laboratori i de camp, han aportat resultats complementaris respecte al coneixement de les respostes microbianes a la sequera. Els resultats de laboratori indicaren que les comunitats microbianes de rius permanents (amb aigua sempre) tenen gran capacitat de descomposició de polisacàrids simples derivats de l'entrada de matèria orgànica autòctona i també de compostos de cel·lulosa i hemicel·lulosa derivats de les entrades de materials al·lòctons del bosc de ribera. Contràriament, en rius intermitents o amb fase seca de llarga durada, es va observar un augment de l'acumulació i la degradació de compostos més recalcitrants com la lignina, probablement a causa de l'acumulació de fulles i de fusta sobre el llit del riu sec. De forma similar, els resultats de l'estudi de laboratori indicaren que els enzims responsables de la degradació de polisacàrids simples es van veure reduïts en condicions de sequera, sobretot en el sediment superficial. A més, l'experiment de laboratori va ressaltar la importància dels moments de precipitació per a la recuperació del funcionament del sediment superficial. La sequera de llarga durada simulada al laboratori va resultar en un augment de la producció de compostos polimèrics extracel·lulars (EPS) en el biofilm per part de les comunitats microbianes per tal d'augmentar la retenció d'aigua i humitat en la matriu d'EPS.

D'altra banda, la biomassa bacteriana i la diversitat total d'aquestes comunitats eren més resistents a les condicions d'escassetat d'aigua que les seves funcions, tot i que la viabilitat cel·lular es va veure molt afectada i reduïda durant el període de sequera prolongada. L'anàlisi de les respostes microbianes en diferents micro-hàbitats va identificar el sediment profund i les acumulacions de fullaraca com els hàbitats amb major resistència a la sequera, probablement degut al manteniment d'un cert grau d'humitat en aquests hàbitats durant la fase molt seca. Aquest fet pot ser que determinés, en aquests hàbitats, una menor producció d'EPS i, a l'inrevés, determinés el manteniment de les activitats enzimàtiques, la biomassa bacteriana i la viabilitat bacteriana en el biofilm en contrast amb el que es va observar al sediment superficial. Des del punt de vista de l'estructura i composició de la comunitat bacteriana, els resultats del treball de camp indicaren que la diversitat estava afectada pels paràmetres ambientals de cada lloc de mostreig i per les característiques de la conca (com serien els usos del sòl i la cobertura de vegetació; per exemple l'ús agrícola i reducció del bosc de ribera afectava en una reducció de la diversitat microbiana).

No obstant això, la composició de la comunitat microbiana observada en condicions de sequera de llarga durada indicava que algunes espècies presents al sediment estaven molt

afectades per l'escassetat d'aigua. Els canvis observats suggerien una transició de la comunitat microbiana aquàtica a una comunitat terrestre. En concret, durant la sequera l'abundància dels grups taxonòmics de Thermoplasmata (archaea) i de Actinobacteria i Bacilli (bacteris) augmentaren, mostrant una gran resistència a l'absència d'aigua. Els arqueus, bacteris i fongs van revelar diferents sensibilitat als canvis hidrològics, on els arqueus van mostrar ser els més sensible seguits pels bacteris, mentre que els fongs actuaven com més resistents a la sequera.

Finalment, les eines moleculars i funcionals utilitzades en el conjunt d'experiments realitzats per caracteritzar la diversitat microbiana total (ADN), activa (ARN) i funcional (CLPP) no van resultar redundants, sinó que aportaven informació complementària en analitzar la dinàmica dels microorganismes en condicions de canvis hidrològics.

En general, hem observat que entre els hàbitats considerats, el sediment superficial és el més amenaçat per la sequera a causa de la irradiació directa i la pèrdua ràpida d'humitat, que afecta de forma important les comunitats microbianes. En global hem observat que els microorganismes que viuen al sediment del llit dels rius són vulnerables als canvis hidrològics que afecten els rius intermitents i això es pot considerar alarmant tenint en compte l'impacte en el funcionament de l'ecosistema i la seva conservació.

1.GENERAL INTRODUCTION

Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.

Marie Curie

This section provides an overview of the background of this thesis

1.1. Intermittent streams (IS): the habits to dry up

1.1.1. Aquatic and terrestrial alternation: IS definition, distribution & hydrology

Natural freshwater bodies which experience a recurrent dry phase and present intermittent discharge are named temporary or intermittent systems (Datry et al., 2016). The peculiar element defining the intermittent streams (hereafter IS) nature is the alternation between flow (wet) and no-flow (dry) periods over time. The term intermittent stream refers to all the temporary freshwater ecosystems, including ephemeral (rivers that flow less than 20% of the time), seasonal (with regular seasonal intermittency) or episodic (those that flow only occurs after rainfall episodes) rivers and streams (Datry et al., 2017a).

In the global landscape, the intermittent streams are not restricted to arid regions but represent more than 30% of the total length and discharge of the global river networks (Acuña et al., 2014; Larned et al., 2010). This estimation was rather conservative and did not include low-order streams (e.g. headwaters because they are difficult to detect and map) which would make up more than 70% of river networks prone to flow intermittency (Datry et al., 2014). For instance, most of the Alpine, Arctic and Antarctic rivers are temporary waters, and the intermittency is also widely spread through United States, Australia, Europe and South Africa (Larned et al., 2010; Sheldon et al., 2010; Snelder et al., 2013). In Mediterranean regions IS are the dominant freshwater type with remarkable diversity including spring-fed karsic rivers and streams, snow-melt and rain-fed headwater streams and braided channel networks (Tockner et al., 2009; Acuña et al., 2014; Skoulikidis et al., 2017). The Mediterranean areas are adapted to natural water scarcity, especially the south-eastern part of Spain belongs to the driest regions in Europe, with a mean precipitation of 120 mm/year (Estrela et al., 2012; Skoulikidis et al., 2017). In this context, future increases in freshwaters intermittency over the biosphere are predicted as consequence of climate change and water (surface and groundwater) abstraction for socio-economic uses, such as agriculture, urban and industry (Döll and Schmied, 2012; Larned et al., 2010; Vorösmarty et al., 2000).

During the last 50 years several perennial rivers became “artificially” intermittent, due to the excessive water abstraction (e.g. Nile, Colorado, Rio Grande). On the contrary, some naturally intermittent rivers have become perennial due to controlled water releases from dams and weirs, agricultural, industrial, and municipal effluent discharges and inter-basin water transfers (Datry et al., 2014; Larned et al., 2010; Steward et al., 2012).

So far, the peculiar intermittent hydrology of intermittent streams is a topic of interest and the study of IS ecology, biogeochemistry and microbiology are expanding our knowledge about their functioning with the final scope to improve their conservation (Datry et al., 2017a). All the intermittent streams are characterized by periods of flow cessation, but the duration, frequency and intensity of these periods can vary widely between systems, depending on regional or local factors (McDonough et al., 2011). Usually, a yearly variable discharge regime of a maximum peak in winter and a minimum flow in summer mark the Mediterranean IS hydrology, being influenced by

the heterogeneity in temperature and rainfall regimes caused by the semi-arid Mediterranean climate (Poquet et al., 2009; Stubbington et al., 2017).
In general, the hydrological transition of wet-to-dry and dry-to-wet phases (Fig. 1a) follows different sequences during which the streambed can be (or not) completely dried and the rewetting can determine the flow resumption or several "false starts" (Fig. 1c) before the surface flow resumes (Fig. 1b; Datry et al., 2017).

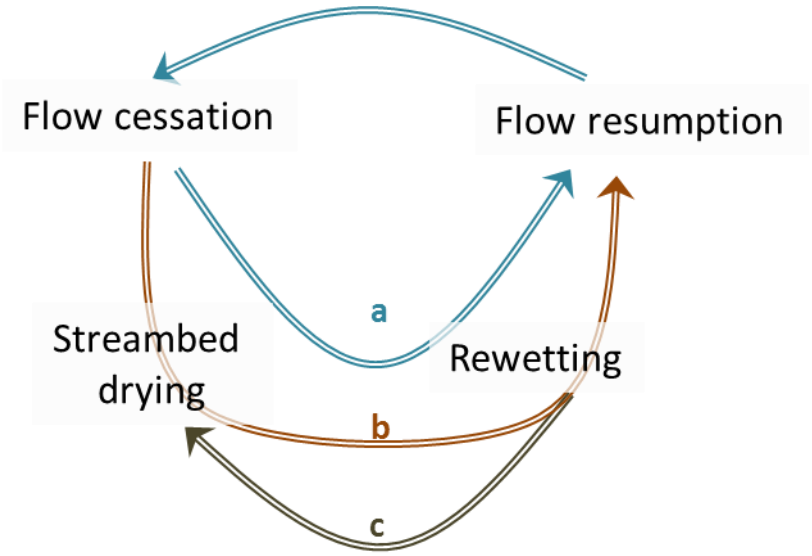

Figure 1. Conceptual diagram representing the intermittent hydrology: (a) alternation of flow and no-flow phases; (b) alternation of flow and no-flow phases with completely dried streambed; (c) alternation of flow and no-flow phases with completely dried streambed and "false starts" before the total rewetting event. This figure is modified from Chapter 2.3 in Datry et al., 2017 Book.

One distinctive feature of IS streams is that when surface flow ceases, surface water can form isolated pools that may be connected by hyporheic flow (Argyroudi et al., 2009; Boulton et al., 1998) but when drying process persist, also the hyporheic flow can cease (Datry et al., 2014). In most of the IS dry and rewetting phases differ in their suddenness, for instance the drying process is usually prolonged and gradual whereas water return is fast and normally coincides with the flow resumption (Lake, 2003). At multiple spatial and temporal extents, the drying and rewetting patterns can occur simultaneously among tributaries or at different sites along the main river path (Costigan et al., 2016; Datry et al., 2014; Stanley et al., 1997). The hydrological fluctuation between expansion, contraction and fragmentation phases identifies the typical patterns of IS hydrology and generates a discontinuous dynamism between terrestrial and aquatic ecosystems (Winemiller et al., 2010). Therefore, aquatic-terrestrial systems are tightly coupled, especially through the soil-sediment continuum hosting the microbiota capable to adapt to dry and wet phases and maintain ecosystem processes.

1.1.2. IS at the catchment scale: connectivity and general functioning

Expansion and contraction of the IS network correspond to the increase of moisture/wet conditions or to the depletion of the water reserves, respectively (Costigan et al., 2016). The three spatial dimensions of the rivers and streams, as longitudinal, lateral and vertical, interact with time and define the water-mediated transfer of matter, energy or organisms (Pringle, 2003). In IS the flow intermittency can disrupt the hydrological connectivity (Fig. 2) in one or more of the three spatial dimensions described above, and in the most extreme scenario this could be found at the same time in the up, mid and low-land reaches (Fig 2; Datry et al., 2017b; Steward et al., 2012).

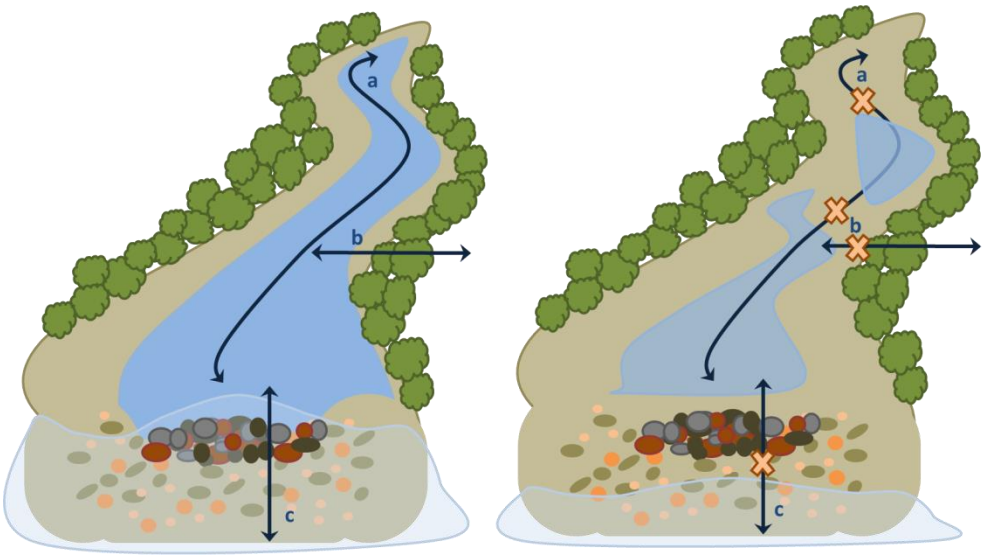

Figure 2. The longitudinal (a), horizontal (b) and vertical (c) connectivity. Interruption of hydrological connectivity is indicated by orange crosses. This figure was modified from Chapter 2.3 in Datry et al., 2017 Book.

Several impairments are observed in system processes (i. e. physical, chemical, and biological) due to a decrease of connectivity at the river network scale (Datry et al., 2014). For instance, the flow cessation (with or without the complete drying of the streambed) alters the longitudinal transport of sediments (as well as other organic compounds and biota) from upstream to downstream sections, induces the lateral isolation of aquatic habitats along the riparian zone and, vertically, determines the disconnection from the hyporheic zone (Datry and Larned, 2008; Nakano and Murakami, 2002). Depending on the severity of hydrological stress, water beneath the dry streambeds may continue to flow through subsurface paths (Stanley et al., 1997) or became hydrologically disconnected between surface habitats and subsurface waters below. This disconnection process reduces up-welling (i.e., subsurface water returning to the stream) and down-welling (i.e., stream water entering the streambed) zones described as key sites for biogeochemical transformations (Boulton et al., 1998). The effects of up-welling zones on microbial streambed biofilms have been related to

the hydrolytic activity linked to the surface water inflow velocity and also presented evidence for microbial participation in river-bottom clogging (i.e., microbial control on hydrodynamics, Battin and Sengschmitt, 1999). The down-welling zones have been linked to hydrologic exchange processes, and especially the subsurface flow paths increased the reach-scale streambed dissolved organic carbon metabolism (Battin, 2000).

The connectivity alterations in IS limit the typical functions of the rivers such as storing, transforming and transferring organic matter and nutrients. These alterations are rather underestimated at the watershed scale in terms of regional and global biogeochemical processes (e.g. organic matter transformation or carbon dioxide efflux among others), since most of these studies are based exclusively on perennial watercourses (Battin, 2000; Benstead and Leigh, 2012). Recent research highlighted that the dynamics of the organic matter and nutrient cycles in IS could greatly vary when compared to those of the perennial rivers, the former being more variable and distinctly pulsed (Datry et al., 2014). As streams dry, large accumulation of leaf litter characterizes the streambed condition for extended periods, terrestrial plants colonize and replace the aquatic macrophytes and algae, and also hypoxic isolated pools can cause rapid release of phosphate and ammonium (Corti et al., 2011; Gomi et al., 2002; McLaughlin, 2008; von Schiller et al., 2011). On the other hand, when flow is resumed a large quantity of organic matter, nutrient and terrestrial plants can be transported downstream depending on the intensity of the rewetting (Corti et al., 2011). Overall, during the rewetting events the organic matter (e.g. all the dissolved organic carbon and nutrients) leaching can determine spikes of solute concentrations becoming important carbon sources for heterotrophic organisms (Bunn et al., 2006; Corti et al., 2011; Jacobson et al., 2000).

1.1.3. Ecological value of IS

Up to now, many studies stood out the importance of IS ecosystems, which are important components of river networks and have notable ecological and social values associated (Acuña et al., 2014; Steward et al., 2012). Ecologically, intermittent streams are considered as intermittent longitudinal reactors and as links between water stored in soils, aquifers, snowpack, glaciers, vegetation and atmosphere systems (Datry et al., 2017a). The flowing network expansion and contraction fluctuations are of paramount importance for the maintenance of the ecological services in IS ecosystems. A wide range of ecosystem services are provided from IS, since during flow and no-flow periods, water, energy, organisms and material are conducted longitudinally and laterally (Acuña et al., 2014). For instance, in cold regions (as alpine, polar or boreal catchments) the melted water from ice and snow flow through IS networks to reach and feed lakes and perennial rivers (Malard et al., 2000; Mcknight et al., 1999). On the other hand, in arid and semi-arid regions, most of the groundwater recharges are mainly fed by temporary stream channels (Izbicki, 2007; Shentsis and Rosenthal, 2003).

In terms of lateral exchanges, IS and riparian vegetation (which provide essential wildlife habitats, forage for livestock, and wood among others) are intimately linked, and nutrients and organisms are moved back and forth between the streambed and the riparian vegetation compartments during stream flow fluctuation. Furthermore, intermittent streams are also considered as flood control systems (Foody et al., 2004) and drains for agricultural and municipal effluents (Cherifi and Loudiki, 1999; Hassan and Egozi, 2001). Floods in IS have historically been used for irrigation (Nabhan, 1979) though the ecological and societal consequences of large organic matter pulses driven by intense rewetting episodes are still unexplored. In the actual scenario of climate change, such abrupt release of organic matter during rewetting will certainly impact the overall greenhouse gas emissions and carbon sequestration of the river ecosystem (Datry et al., 2017a; Steward et al., 2012).

1.2. Microbial life in intermittent streambed

1.2.1. Intermittent streambed: the relevance of the habitat patchiness

The temporal instability of flow conditions determines the spread of habitats formation within the streambed (longitudinally, vertically and laterally), at any time and depending on the hydrological, climatic and geomorphological features (Steward et al., 2012). In particular, the decrease of surface water derives to a mosaic of habitats and microhabitats suitable for both aquatic and terrestrial biota and microbiota (Fig. 3, Costigan et al., 2016). The shape and features of intermittent streambeds and their boundaries are constantly changing as the stream contracts or expand the flow condition. Consequently, distinct communities can occupy different spots formed within the streambed and increase the overall biodiversity that can compensate the diversity decrease observed during no-flow conditions (Larned et al., 2010; Stanford et al., 2005). Therefore, the intimate linkage existing between streams and the surrounding terrestrial environment is reflected in the streambed compartment.

Commonly, during the drying phase, a part from the isolated moist zones and the dry bare ground spots, the intermittent streambed presents some habitats better conserving humidity, such as leaf litter packs (on the surface or buried within the sediment), woody debris and algal mats (Romaní et al., 2017; Steward et al., 2012). Many terrestrial organisms (from arthropods to mammals) find refuges in the drying streambed (Corti et al., 2011; Steward et al., 2012) while many semiaquatic and aquatic organisms (e.g. insects, invertebrates or some fishes and amphibians) can only occupy isolated pools that persist during dry periods (Sheldon et al., 2010).

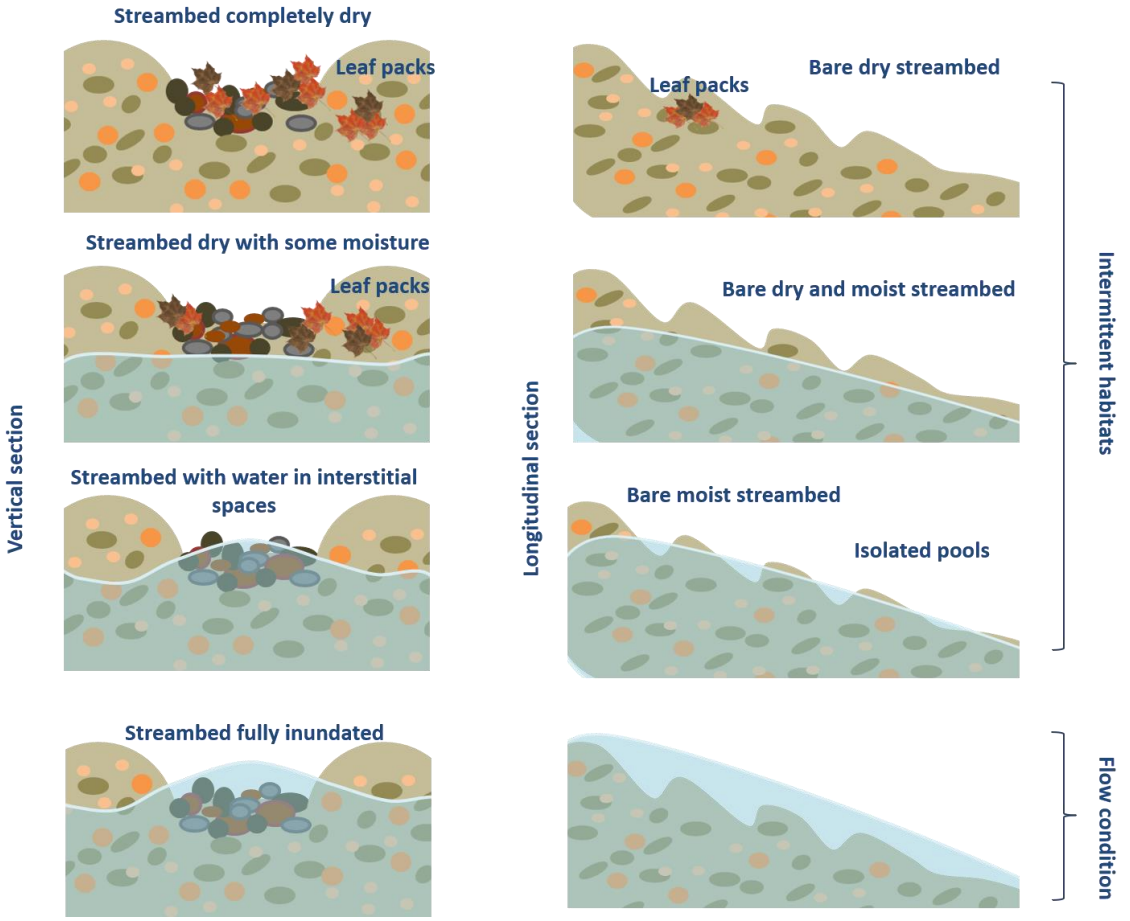

Figure 3. Conceptual diagrams identifying streambed vertical (on the left) and longitudinal (on the right) sections and micro-habitats formed (as bare dry or moist sediment, leaf packs, isolated pools) under distinct hydrological conditions. This figure was modified from Costigan et al., 2016).

Therefore, the major relevance of the mosaic of habitats created under the fluctuation of stream flow, and especially during the drying phase, consists in offering refuge to the biota and microbiota (as prokaryotes and eukaryotes) colonizing the stream sediment (Romaní et al., 2017). These refuges allow microbes to cope with the harsh conditions mainly consisting in water scarcity and direct radiation, but also in the limitation of nutrients diffusion and organic matter availability. Given the importance of the streambed microbiota being the engine ensuring key ecosystem's functions, the streambed habitats will act as microbial refuge permitting to maintain microbial-mediated stream processes such as nutrients and carbon cycling. Remarkably, the highly variable nature of microhabitats created within the streambed under the intermittent hydrological cycle could lead to patchy distributions in microbial taxa and changes in ecosystem functions over space and time, enhancing waves of microbial diversity and overall system functioning (Datry et al., 2017a; Romaní et al., 2017; Steward, 2012; Vadher et al., 2017).

Humid hyporheic zones and leaf packs buried into the sediment allow the microbial survival when the desiccation persists (Febria et al., 2012; Timoner et al., 2014a; Ylla et al., 2010). Although the strong fluctuations in the oxygen level can greatly impact some bacterial species rather than archaeal ones, many microbial communities colonize the moist hyporheic sediment to survive the drying phase (Angel et al., 2013; Timoner et al., 2014b, 2012). The hyporheic zone and the leaf packs are important habitats also for the conservation of the aquatic fungal communities during the drying processes, although their sensitivity to drought and reduced oxygen conditions make them use strategies including the colonization of surrounding terrestrial habitats (amphibian fungi; Bärlocher et al., 2006; Cornut et al., 2010; Sudheep and Sridhar, 2012). Contrariwise, the isolated and anoxic drying pools determine the selection of some bacterioplankton species and a wide range of protozoa especially adapted to harsh conditions including abrupt fluctuations in dissolved organic matter (Fazi et al., 2013; Medeiros et al., 2009; Schlief and Mutz, 2011). The isolated pools conditions have opposing effects for fungal species development since i) low pH and oxygen would disadvantage fungi, while ii) high conductivity and temperature would favour their growth (Canhoto et al., 2016; Medeiros et al., 2009; Mora-Gómez et al., 2015).

1.2.2. Microbial biofilm inhabiting the streambed

The type of microbiota coexisting in the IS streambeds includes aquatic, semi-aquatic and desiccation-resistant or more terrestrial taxa. The continuous alternation between wet and dry conditions influences the streambed microbial communities that may assemble in a stable biofilm structure within the sediment particles (Pusch et al., 1998).

Stream sediment biofilm, defined as aggregates of microorganisms embedded with self-produced extracellular polymeric substances (EPS, Flemming et al., 2016), are in general more heterotrophic than those colonizing the rocks surface (Fig. 4, Romaní and Sabater, 2001), with higher contributions of bacterial and fungal communities (Brablcová et al., 2013; Timoner et al., 2014a). Although the surface streambed biofilm matrix hosts some algae, the larger community is composed by bacteria and fungi groups and this is mainly due to the type of substrate colonized. For instance, stream sediment is generally less stable than rocks and it captures lower solar radiance, due to the continuous surface particles-movement under flow condition, these substrate characteristics are rather unfavorable conditions for the algal community to develop (Romaní and Sabater, 2001). Overall, both superficial and hyporheic sediments present assemblages of microbes organized in biofilms that contribute to ecosystem processes such as biogeochemical fluxes and organic matter decomposition (Battin et al., 2016). The microbial life within streambed biofilms is regulated by complex interactions between prokaryotes and eukaryotes. For instance, prokaryotes feed on algal exudates and on dried and decaying algae whereas both prokaryotes and fungi mainly degrade the organic matter and so transfer energy and materials to higher trophic levels, such as protozoans and invertebrates through predation (Flemming et al., 2016; Flemming and Wingender, 2010). Furthermore, thanks to the extracellular polymeric matrix the biofilm humidity is maintained during the drying phase (Roberson and Firestone, 1992)

representing a perfect microbial refuge against the hydrological fluctuation of IS. This EPS matrix also contributes to enhance carbon and nutrients capture, giving physical resistance against abrupt changes in the sediment stability (e.g. flood and desiccation) and providing protection to toxicants (Flemming et al., 2016).

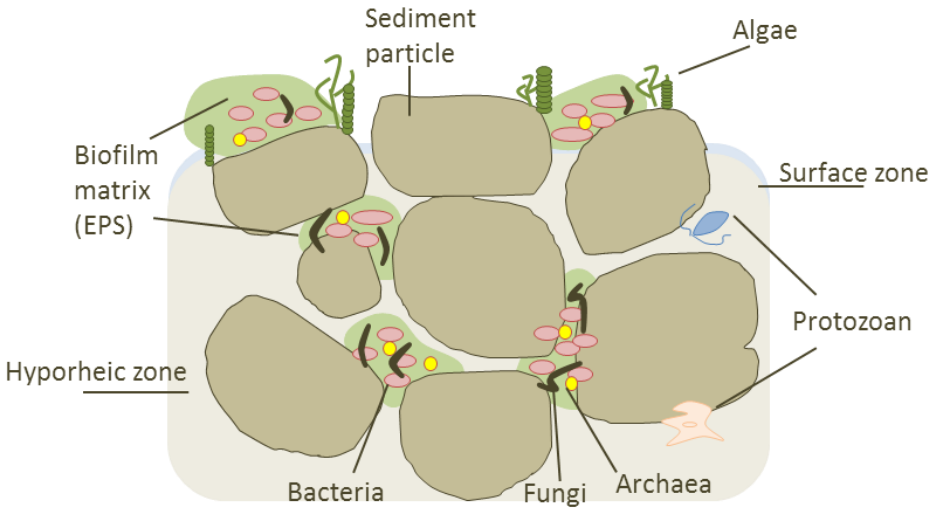

Figure 4. Conceptual diagram describing sediment biofilm. This figure was modified from Battin et al., 2016

The specific microbial diversity colonizing the streambed results in a jungle of microorganisms adapted to the light, oxygen and hydrological conditions of the media. Recently, next generation sequencing techniques (e.g. 16S rRNA gene sequencing) have permitted to better characterize microbial communities' diversity (Bengtsson et al., 2018; Romaní et al., 2014; Zeglin, 2015). In IS ecosystems, bacteria are the most studied communities compared to those of fungi, archaea and protozoa (Weitere et al., 2018; Zeglin, 2015). In the case of bacteria, *Proteobacteria* and *Bacteroidetes* tend to dominate the streambed biofilms, mainly during the wet phase (Freixa et al., 2016; Timoner et al., 2014b; Zeglin, 2015). Contrarily, the dry condition is often dominated by *Actinobacteria* and *Firmicutes* who possess specific characteristics (e.g. Gram-positive cell-wall type, endospore-forming genera) permitting to survive during desiccation in IS (Fazi et al., 2008; Klappenbach et al., 2000). So far, little is known about archaeal and fungal communities inhabiting the intermittent streambed and their responses to the hydrological conditions. For instance, archaea ammonia oxidizers (AOA) colonizing streambed are remarkably involved in the in-stream nitrogen cycling, and they revealed no major changes across hydrological conditions or sediment layers (Merbt et al., 2016). In the case of fungi, most of the research described those communities colonizing the leaf litter present on or into the streambed surface (Abril et al., 2016; Mora-Gómez et al., 2015; Artigas et al., 2009, 2011). Microbial heterotrophs (mainly fungal group) colonization of submerged leaf litter and organic matter use in Mediterranean type streams are modulated by

environmental conditions, especially the hydrological variability (Artigas et al., 2011). For instance, during autumn season high current velocities may fragment and erode leaves, stimulating leaf breakdown, but at the same time, floods may modify the structure and activities of attached microbial communities (Artigas et al., 2009). In the case of leaf litter accumulated on the streambed surface, a recent research reported that the emersion time (usually identified as summer or dry period) can determine the reduction of fungal biomass, changes of the community enzyme activities and structure, in terms of both diversity and composition (Mora-Gomez et al., 2018). Specifically, *Clavariopsis aquatic*, *Lemonniera aquatic* and *Articulospora tetracladia* seemed to be favoured by the emersion time whereas the contribution of the species *Dimorphospora foliicola*, *Tricladium chaetocladium*, *Tetrachatum elegans*, *Alatospora pulchella, Lunulospora curvula* and *Clavatospora longibranchiata* was affected negatively by the dry condition (Mora-Gomez et al., 2018).

1.2.3. Metabolism of the streambed microbial communities

In general, streambed bacteria account for about 60 % of total community respiration in streams and are responsible for most of the metabolic activity (Marxsen, 2006). Within the hyporheic zone, the exchange of water, nutrients and biota during the wet phase influences stream water quality and organic matter degradation (Boulton et al., 1998; Marmonier et al., 2012; Rulík and Spáčil, 2004).

The overall IS metabolism is mainly driven by streambed prokaryotes and eukaryotes (in particular fungi and protozoans) (Gao et al., 2005; Risse-Buhl et al., 2012). In the benthic compartments the occurrence of reduction and oxidation processes favour the prokaryotes-mediated organic matter decomposition (Gao et al., 2005) from flowing (wet phase) or interstitial (also during dry phase) water or from particulate material (Adams et al., 2015; Romaní et al., 2012). Recent studies reported that the overall leaf decomposition is heterogeneous during flow fragmentation, which has implications related to the dissolved organic carbon utilization that should be considered in future regional carbon budgets (Abril et al., 2016; Foulquier et al., 2015). Furthermore, leaf decomposition rates and the activity of some extracellular enzymes (e.g. β-glucosidase, cellobiohydrolase and phosphatase) developed by both bacterial and fungal communities, can be gradually reduced with larger dry phase duration (Mora-Gómez et al., 2018). Desiccation can reduce microbial biomass and fungal sporulation and also enhance the presence of taxa that may better adapt such as those capable to regulate intracellular osmotic pressure or turn to dormancy (Timoner et al., 2012; Mora-Gómez et al., 2018). However, differences may occur among groups because the thicker cell wall of fungi can confer better resistance to desiccation than bacteria (Foulquier et al., 2015; Schimel, J., 2007). In the case of protozoans their ecosystem functions are mainly related to predation of bacteria (Norf and Weitere, 2010; Risse-Buhl et al., 2012). The competition for food among protozoans determines that bacteria constitute a valuable and nutritious resource (Hakenkamp and Morin, 2000). Furthermore, protozoans can cope with the hydrological phase's alternations thanks to the high diversity in their assemblages which provide genotypes well adapted to different conditions and high tolerance to changes in salinity, oxygen and temperature

((Finlay and Esteban, 2009; Norf et al., 2007), determining peaks of activity during fragmentation and isolated pools formation.

1.3. Shrinking and drying: assessing the fragility of the IS to global change

1.3.1. Drought issue: at the interface between aquatic and terrestrial systems

Drought is a recurring feature of all climatic regimes and among the most damaging and least understood of natural hazards (Gornall et al., 2010; Lesk et al., 2016). It is a natural phenomenon that can occur in high or low rainfall areas, differently to aridity, which is a permanent feature of the climate and is restricted to deserted low rainfall areas (Wilhite, 2000). Frequency and intensity of droughts are predicted to increase worldwide in the next years due to global climate change (as temperature increase) and increasing demands in potable water (Lesk et al., 2016; Naylor and Coleman-Derr, 2018).

In Europe the severity and frequency of meteorological and hydrological droughts have increased, in particular in south-western and central Europe. Between 2006 and 2010 the 15 % of the European territory and 17 % of the European population, on average, have been affected by meteorological droughts each year (EPA www.epa.gov). Available studies project large increases in the frequency, duration and severity of meteorological and hydrological droughts in most of Europe over the 21st century, except for northern European regions. The greatest increase in drought conditions is projected for southern Europe, where it would increase competition between different water users, such as agriculture, industry, tourism and households (EPA www.epa.gov, map Fig. 5). In this context the arid and semi-arid and climate areas are among the most prone to drought and therefore the most exposed to lose integrity of river networks functioning (Bonada and Resh, 2013; Giannakopoulos et al., 2009; Prudhomme et al., 2014).

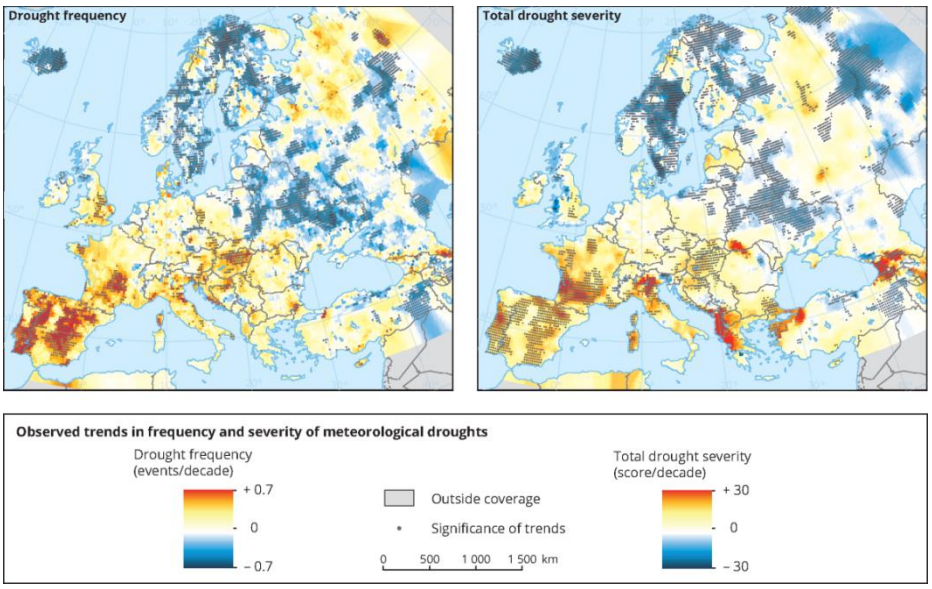

Figure 5. Drought frequency and severity maps from EPA (Environmental Protection Agency), www.epa.gov

In the case of IS systems, the historical repetition of wet and dry phases could favour the adaptation of the biota and microbiota ensuring the conservation of the whole system functioning to a certain extent, but the consequences of prolonged periods of water deficit need further investigations. Recent studies reported that IS systems depict higher resilience to climate change consequences than the perennial freshwater ecosystems (Botter et al., 2013). Rivers with low mean discharges are described theoretically and empirically as more resilient in that they hold a reduced sensitivity to climate fluctuations (Botter et al., 2013). Similar to soil systems, the overall “stasis” of the microbiota-mediated system processes inhabiting IS systems under stressful and prolonged drought was suggested less damaging than the consequent rewetting for the terrestrial microbiota (Kieft et al., 1987; Schimel et al., 2010). Contrarily, perennial, temperate, low-order streams are predicted to become intermittent and their metabolism can change due to irregular droughts (Zlatanović et al., 2017). Strong effects of droughts on microbial structure and functions of temperate streams have been described recently (Marxsen et al., 2010) revealing the importance of stream management under a changing climate to mitigate the future impact of unpredictable droughts in temperate ecosystems.

Many similarities can be found between dry streambed and soil systems functioning. The long duration of the dry phase and the reduced inundation frequency mainly enhance the acquisition of soil characteristics (for instance, the larger abundance of Gram-positive species in the case of bacteria, or the survival of fungal species with amphibian-nature) by the intermittent aquatic system which then becomes “terrestrialized” (Arce et al., 2019; Elosegi et al., 2010; Morandi et al., 2014; Mori et al., 2017). Actually, dry streambeds not only present similarities to the surrounding soils for the external features or the microbiota diversity but especially for their biogeochemical responses to water pulses (Austin et al., 2004; Fossati et al., 1999). Among others, the pulsed increased carbon mineralization and emissions of CO_2 from dry streambed when abrupt precipitations rewet the sediments is a typical process that has been previously well described in soils and known as the "Birch effect" (Birch, 1958; Birch and Griffiths, 1961; Gallo et al., 2014; Marcé et al., 2019). Only recently the estimation of C fluxes from dry inland waters has been taken into consideration (von Schiller et al., 2014). Making profit from soil science knowledge that already considers some semi-aquatic habitats that recurrently fall dry (e.g. peatlands) would offer advantages of all technical and theoretical developments available to measure and control these emissions from intermittent streams (Gómez-Gener et al., 2016; Marcé et al., 2019). Indeed, CO_2 emissions from dry streams and rivers could have potential relevance considering the high CO_2 emission rates measured in these systems and the large area they cover globally (Marcé et al., 2019). High CO_2 (and low CH_4) emission rates have been measured in the dry sediments of other types of inland waters, including ponds (Obrador et al., 2018), reservoirs (Gómez-Gener et al., 2015) and lakes (Koschorreck, 2000).

Drying stress also increases the degree of similarity among the microbial communities of aquatic and terrestrial systems, which could be driven by dispersal and colonization from adjacent terrestrial systems or through the adaptation/selection of taxa responding to specific dry-habitat requirements (Febria et al., 2015, 2012; Monard et al., 2016; Sabater et al., 2016; Timoner et al., 2014b). Recent research from soil systems revealed that microbiota usually well cope with the initial drying process because certain bacterial and fungal species are mainly drought tolerant and they can maintain low rates of activity and growth (Schimel et al., 2010). When prolonged dry periods become stressful, each biological activity becomes energetically expensive and microbes experience direct physiological stress due to water restriction and resource limitation. Contrarily, under prolonged desiccation microbes may experience reduced predation pressure (Görres et al., 1999), since protozoa require water-filled pores to forage (Stefan et al., 2014). In IS streambed, generally bacterial and fungal biomass and activity are reduced during the drying processes, protozoans activity highly depend on the interstitial water whereas archaea are better suited to remain active under the unusual prolonged anoxia and changes in organic matter quantity and quality (Amalfitano and Fazi, 2008; Tzoraki et al., 2007).

Therefore, the biogeochemical and microbial similarities already observed between soils and dry streambeds suggested that the intermittent aquatic ecosystems could reflect a first stage of a terrestrial system originated from alluvial deposits (Arce et al., 2019; Grimm et al., 2003), especially when prolonged drought periods are present.

1.3.2. Coupled / Uncoupled microbial structure and function responses to drought

Changes in microbial functions under hydrological constrains can be coupled or not with shifts in microbial structure. In microbial ecology the common principle that the structure determines the function is usually unsolved and studies on microbial functional-diversity relationships in IS are still scarce. The great resistance of microorganisms to (repeated) disturbances, such as hydrological changes, suggest their capacity to remain unaltered revealing weak coupled or uncoupled patterns between community function and structure (Frossard et al., 2012; Gibbons et al., 2014; Tranvik et al., 2005). Specifically, a microbial community subjected to a disturbance can manifest functional plasticity, maintaining its composition and change its functions, or functional redundancy, altering its composition but not the community functions (Allison and Martiny, 2008).

Microbial functional stability is more often compromised compared to that of structure when different hydrological conditions are present. Despite flow state alternations change the microbial heterotrophs capabilities of organic matter degradation, by different utilization of extracellular enzymes by streambed communities, the community composition can be conserved manifesting functional plasticity (Freixa et al., 2016; Marxsen et al., 2010; Romaní et al., 2013; Timoner et al., 2012). Despite most of the functional changes are not coupled with structural changes, other studies observed differences in the community composition or diversity but not

in functions suggesting a high functional redundancy (Frossard et al., 2012; Lear et al., 2014; Wagner et al., 2014). In the case of fungal communities contrasting results are presented in the literature, even if most aquatic fungal species exhibit a wide range of enzymatic capacities revealing functional redundancy among species in leaf-associated assemblages presented in IS (Bruder et al., 2011; Foulquier et al., 2015; Mora-Gómez et al., 2015; Schlief and Mutz, 2011).

The streambed microbial communities inhabiting the IS systems and subjected to hydrological fluctuations may reveal either functional plasticity or redundancy and thanks to these traits they can adapt to hydrological disturbances. The overall microbial resistance (as the ability to cope with stress and remain unaltered) and resilience (as the ability to quick recover and return to its original state) capacities to cope disturbances can derive to the stability of the community (Allison and Martiny, 2008; Shade et al., 2012) under different intensity of disturbances.

At present the dynamics of microbial structure/function response to drought stress in streambed of intermittent streams is not yet clearly deciphered. Accordingly, testing where and whether there is a limit of the streambed IS microbial adaptation beyond which the community would be completely altered (in terms of structure and function) by prolonged drought or abrupt rewetting, was the main interest driving the present thesis.

2. RESEARCH objectives

The history of science knows scores of instances where a researcher was in the possession of all the important facts for a new theory but simply failed to ask the right questions.

Ernst Mayr

This section outlines the main and specific research objectives.

The main objective of this thesis is to analyse changes in the structure, function and composition of microbial communities inhabiting intermittent streambeds when submitted to hydrological stress characterized by prolonged dry periods, punctual storms and rewetting events. We monitored the evolution of archaeal, bacterial, and fungal communities inhabiting surface and hyporheic sediments and buried leaves to hydrological stress using laboratory and field experiments.

The specific objectives to reach this main objective are the following:

From the laboratory approach:

- To measure whether the effects of long-term drought (5 months) on microbial biomass and function were distinct depending on the habitat (surface sediment, hyporheic sediment, and leaf packs) (Chapter 1). We expected that the microbial biomass and functions would be strongly reduced in surface sediments, being more directly exposed to long-drought effects. The damper hyporheic sediment and leaf packs would act as microbial refuges, leading microbial structure and function to be less sensitive to long-term drought.
- To investigate the role of flash storms in alleviating long-term drought effects on microbial biomass and function and test whether the storms were differentially affecting the distinct habitats (surface sediment, hyporheic sediment, and leaf packs) (Chapter 1). We suggested that flash storms would promote microbial resilience in particular from the most directly impacted surface sediments.
- To describe and quantify the effects of rewetting on the recovery of microbial biomass and function, and test whether the rewetting was differentially affecting the distinct habitats (surface sediment, hyporheic sediment, and leaf packs) (Chapter 1). We expected that microbial structure and especially function would recover during rewetting.
- To investigate how the taxonomical composition of archaeal, bacterial and fungal communities inhabiting different streambed habitats are affected by hydrological stress (including long-term drought, flash storms, and rewetting) (Chapter 2). As for microbial biomass and function, we expected that microbes inhabiting the surface sediment (as opposed to the other streambed habitats, i.e., the hyporheic zone and buried leaves), would respond more strongly to long-term drought due to the more direct exposure to desiccation effects. Besides this, among the microbial communities, we supposed that, given the specific molecular traits of fungi and the archaeal capacity

of inhabiting extreme environments, fungi and archaea would be the most resistant groups to drought.

- To evaluate the relationships between the different microbial diversity matrices from streambed communities (DNA and RNA based microbial diversity and matrix based on community level physiological profiles), calculated for the eukaryotic and prokaryotic streambed components submitted to hydrological stress (Chapter 3). Correlations between RNA diversity matrices and functional fingerprint were expected for prokaryotes and eukaryotes and between the two communities because of the potential interaction.
- To test whether the distinct diversity matrices show a differential sensitivity to the experimental factors considered (hydrological conditions, sediment depth and time) (Chapter 3). Separation between total (DNA) and potentially active (RNA) diversity pools were expected, especially under extreme hydrological conditions where DNA matrices were supposed to be more conservative while RNA ones more sensitive to changing environmental conditions.
- To assess to which extent the diversity variability is explained by the environmental and functional variables such as water content and extracellular enzyme activities, respectively (Chapter 3). Water content was supposed an important modulator of the entire community's response. On the other hand, RNA-based prokaryotic matrix was expected to be more correlated to the labile (prokaryotes) vs recalcitrant (eukaryotes) organic compounds.

From the field approach:

- To elucidate whether and to which extent the hydrological history (e.g. number of dry/wet days) influences the microbial diversity, composition and activity of sediment microbial communities in Mediterranean intermittent streams (Chapter 4). In sites with long dry-phases, we expected a decrease of microbial labile carbon degradation capacities but an increase of recalcitrant material utilization accompanied by a transition of microbial community diversity and composition to a soil-like microbial community.
- To determine the relative contribution of catchment characteristics (riparian vegetation, organic matter and water content, and land use) versus hydrology on the structure, diversity, and activity of streambed microbial communities in Mediterranean intermittent streams (Chapter 4). We hypothesized that the microbial

functions would be more responsive to hydrology than structure, and mainly influenced by the organic matter and water content in sediments considered as major environmental forces influencing microbiota activities.

3. METHODS

Overview

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This section outlines the methods and study areas used for the development of this thesis. Basic information on the study sites and main methodological procedures are shortly described, while specific details are included within each Chapter.

Different techniques have been applied to monitor the structure, diversity and activity of streambed microbial communities and to achieve the objectives of this thesis. The main methods used in the laboratory and field studies are summarized in this section, while details on experimental designs, technical procedures and sampling strategies corresponding to specific objectives are directly reported in the chapters. C*hapters 1, 2* and *3*correspond to the laboratory experiment, whereas *Chapter 4* corresponds to the field study. Both the laboratory and field experiments monitor heterotrophic microbial communities inhabiting different streambed microhabitats of intermittent Mediterranean rivers. Specifically, the laboratory experiment was conducted with sediment columns microcosms filled with streambed sediment from the Santa Llúcia de Puigmal stream, affluent of the Fluvià river (*Chapters 1, 2* and *3*). The field experiment was conducted across 37 upstream sites that belong to nine watersheds corresponding to the main rivers of the north-eastern part of the Iberian Peninsula (*Chapter 4*).

Methods

Microbial community structure, biomass and viability

- *Extracellular polymeric substances (EPS) from* the sediments were extracted using cation exchange resin (Romaní et al., 2008). After the extraction of EPS, the polysaccharide content was measured by the phenol-sulphuric acid assay (Dubois et al., 1956) (details in *Chapter 1*).
- *Organic matter* content was measured as Ash-Free Dry Weight (AFDW). Sediment was first dried (placed at 70 ºC for 72 h) and then combusted (at 450 ºC for 4 h) using a muffle furnace (details in *Chapters 1, 4*).
- *Water sediment content* was calculated as percentage of water loss (%) between fresh and dry weight (details in *Chapters 1, 3, 4*).
- *Grain particles size* distribution was determined on fresh sediment samples, treated with H_2O_2 (10% volume) to remove organic matter, and later disaggregated and dispersed ultrasonically with pyrophosphate. Sediment particle fractions up to 2 mm were determined by sieving, while determination of fractions below 2 mm was performed using a Beckman-Coulter LS230 laser (details in *Chapter 1*).
- *Chlorophyll-a* content in sediment samples was used as a proxy for algal biomass. It was extracted with 90% acetone and concentration was determined spectrophotometrically following the protocol of Jeffrey and Humphrey (1975) (details in *Chapter 1*).

- *Fungal biomass* in sediment samples was estimated from ergosterol content by solid-phase extraction and concentration (Gessner and Schmitt, 1996). Ergosterol was detected and quantified using high-pressure liquid chromatography (Waters Inc., Milford, MA, USA) at 282 nm absorbance (details in *Chapter 1*).
- *Bacterial density* was estimated on sediment samples extracts by flow cytometry (FACSCalibur, Becton Dickinson) using Syto 13 stained cells (details in *Chapters 1, 4*).
- *Bacterial cell viability* corresponding to the abundance of live against dead bacteria were estimated using the LIVE/DEAD viability kit (Invitrogen Molecular Probes, Inc.) based on the double-stain of Syto 9 and propidium iodide (Freese et al., 2006) and posterior counting by flow cytometry (FACSCalibur, Becton Dickinson) (details in *Chapter 1*).

Microbial community *functions*

- *Potential extracellular enzyme activities* in sediment samples were analysed fluorometrically using methylumbelliferyl (MUF) substrates or colorimetrically using L-3,4-dihydroxyphenylalanine (L-DOPA)) (Romaní et al., 2012) (details in *Chapters 1, 3, 4*).
- *Respiration activity* in sediment samples was measured through Resazurin assay modified from Haggerty and colleagues (Haggerty et al., 2010). The resazurin (7-Hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt, RAZ) method consists in using a redox dye (blue in oxidised state) that indicates the respiratory activity of microorganisms (turns pink when reduced) (details in *Chapter 4*).
- *Carbon level physiological profiles* (CLPP) were analysed by incubation of sediment extracts inside of the Biolog EcoPlates (Biolog Inc., Hayward, CA, USA) at 20ºC in dark conditions for one week. Optical density in the plates was read every 24h at 590m using a microplate reader (details in *Chapter 1, 3, 4*).

Microbial community composition

- *DNA was* extracted from sediment samples with the FastDNA™Spin Kit for Soils (MP Biomedicals, Irvine, CA) while RNA was extracted using the RNA PowerSoil® Total RNA Isolation Kit (MO BIO Laboratories, Inc.). Details in *Chapter*s *2, 3* and *4*.
- *Illumina MiSeq* technology was applied as sequencing strategy. Details on the primers used for each type of microbial community (bacteria, archaea and fungi), on the bioinformatics procedure followed and on the community structure determination

(e.g. Shannon-Wiener and Richness diversity indices) are specified in each study (*Chapters 2, 3, 4*).

All DNA and RNA sequences generated from this thesis have been deposited in the Short Read Archive (SRA) of the National Centre for Biotechnology Information. Details on the accession number linked to each study are presented in the related Chapters.

Environmental variables

- *Physicochemical parameters* (pH, conductivity, temperature and dissolved oxygen) in waters from the laboratory and field experiments were analysed in-situ using portable probes (WTW, Weilheim, Germany) (*Chapters 1, 4*).
- For the field work, the *hydrology* of the different studied sites was characterized through temperature and pressure transducers (Solinst Levelogger Gold Model 3001 and Solinst Barologger Gold Model 3001, Solinst Ltd, Georgetown, ON, Canada), and temperature data loggers (ACR SmartButton Logger, MicroDAQ) installed one year before sampling (*Chapter 4*).
- The percentage of streambed shadow cover (e.g. <10% unshaded, 10-50% highly illuminated, 50-80% shaded with light spots, >80% totally shaded, considered length 100 m) and riparian vegetation coverage (e.g. the presence of trees, shrubs or small bushes was considered in percentage, from <25% between 25-50%, 50-75% and >75%, for the first 10 m width of riparian vegetation) were measured in situ following a field-assessment procedure (*Chapter 4*).
- Major land-uses (urban zones, agriculture and forest areas, Corine Land Cover 2012, https://www.eea.europa.eu/data-and-maps/data/clc-2012-raster) have been calculated as percentage for the whole stream basin and just for the adjacent 1 km upstream site's catchment (i.e. the intersect between the entire catchment and a buffer radius of 1 km centred on the sampling point) (*Chapter 4*).

Study sites

Laboratory experiment

The laboratory experiment was performed using sediment collected from Santa Llúcia de Puigmal stream, affluent of Riera de Bianya, a headwater tributary of the Fluvià River (42.217637N, 2.401402E). Fluvià river is 97 km long and drains a 990 km^2 catchment covered with mixed forests (78%), and agricultural (19%) and urban (3%) areas. The sediment columns (25 cm height × 15 cm diameter) were filled with homogenised sediment on site and directly transported to the laboratory (Figure 1).

Field experiment

A total of 37 sites between permanent and temporary streams distributed across the NE Iberian Peninsula were selected for the field study (Figure 1). These sites belong to nine main rivers flowing across Catalonia: Muga, Fluvià, Ter, Tordera, Llobregat, Francolí, Foix, Besós and Ebro rivers.

The sampled stream sections, ranging between the 2^{nd} and 5^{th} order, had a gradient of flow regimes ranging from ephemeral to permanent. The study sites were characteristic of mid-mountain altitudes and all of them had a Mediterranean climate characterized by a distinct warm and dry summer period (scarce precipitation occurring primarily in the spring and autumn) and a mild winter.

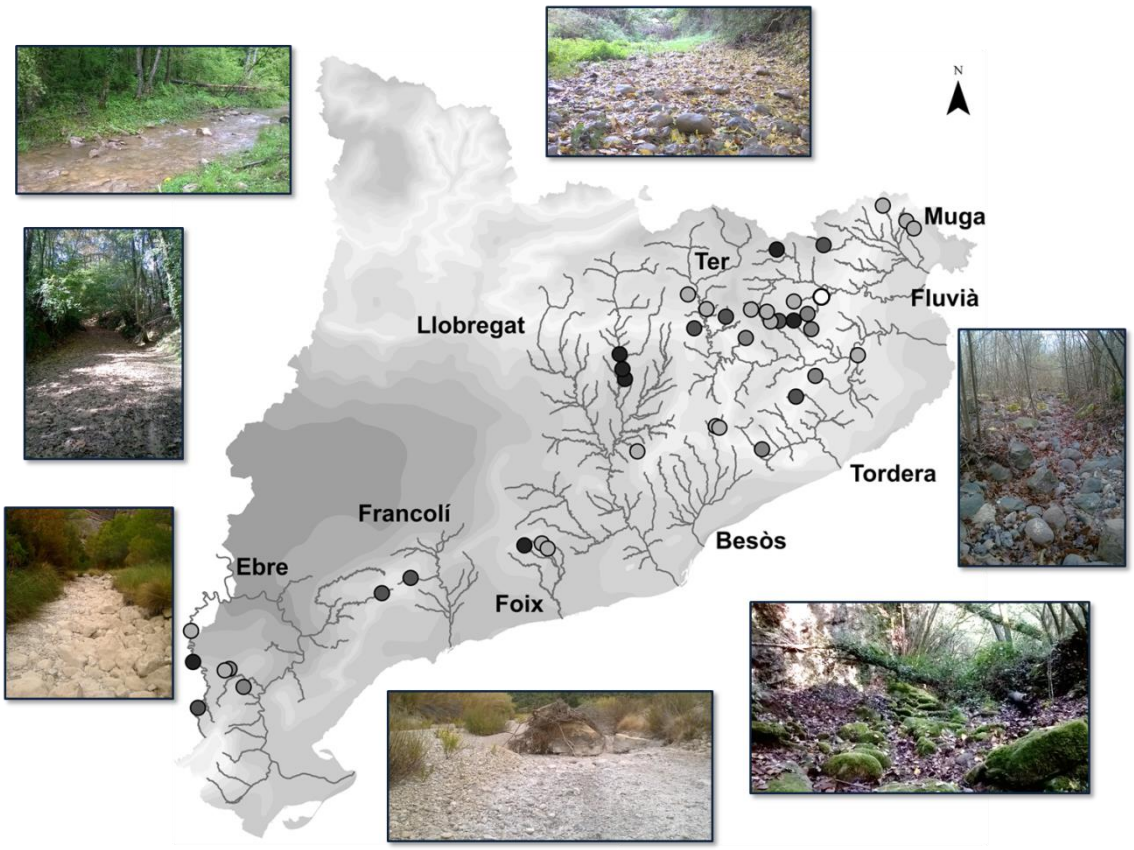

Figure 1. Stream sites sampled for the laboratory (white circle) and field (grey scale circles) studies, among Catalonia. The pictures give an idea about the diversity of streambed considered.

4. RESULTS

The most outstanding feature of life's history is a constant domination by bacteria.

Stephen Jay Gould

This section is structured in four chapters that constitute the main body of the thesis. Supplementary information is provided in the Annex section, at the end of the document.

CHAPTER 1

"Key role of streambed moisture and flash storms for microbial resistance and resilience to long-term drought"

This Chapter was published as:

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OVERVIEW

Spatial and temporal widening of drought periods together with occurrence of flash storm events are consequences of global change affecting temporary stream ecosystems. Streambed heterotrophic microbes and the key biogeochemical processes they carry on could be endangered by the strengthening of drought episodes.

Here, we performed a 165-day experiment through 12 streambed sediment columns to study heterotrophic microbial functional and structural responses to long-term drought. Two sediment depths (surface and hyporheic) and leaf litter were monitored under three treatments, i.e. Control (maintained in wet, pool-like conditions), Dry (5 months of drought), and Dry-Storm (5 months of drought including two flash storms). All treatments were then followed by rewetting.

Surface sediment followed by leaf litter was the most affected by the long-term drought as shown by the reduction of polysaccharidic enzyme activities and litter decomposition rate, although lignin decomposition was not affected. This resulted in a greater use of recalcitrant compounds which might be due to reduced labile organic matter sources and the greater resistance of fungi than bacteria to drought. Moreover, in surface sediment, bacterial viability and algal biomass were reduced while the production of extracellular polymeric substances was intensified with dryness, suggesting its potential role as survival strategy.

Hyporheic sediments appeared more resistant to long-term drought and this might be linked to its slightly higher water content (2.5%) than the surface (0.5%) during drying together with a greater content of fine material that allowed fungi and bacteria to survive and extracellular enzyme activities to be maintained. Microbial resilience to long-term drought in sediment and leaf litter was promoted by the flash storms as shown by the fast recovery of enzyme activities, bacterial viability and leaf litter decomposition rate in the Dry-Storm treatment, once rewetted. Storms might liberate previously occluded carbon sources that fuel the fast-microbial reactivation as well as providing sediment moisture for microbial survival.

Our results highlight that long-term drought may compromise stream biogeochemical processes linked to organic matter decomposition and that microbes are highly sensitive to minimal water content changes. Thus, long-term drought consequences could be mitigated by occasional precipitations or by natural streambed moisture.

BACKGROUND

Arid climatic conditions are predominant in Mediterranean areas with intermittent, ephemeral and episodic lotic waters being common (Skoulikidis et al., 2017). In recent decades, severe shortages of surface and groundwater supply together with the climatic

pressures have lengthened the duration and strengthened the intensity of drought, transforming perennial rivers to temporary freshwaters (Datry et al., 2014) and enhancing their terrestrialization process (Janvier, 2010). Here we define terrestrialization as drought intensification and decreasing groundwater which causes the transformation from aquatic to terrestrial ecosystems. This process might accelerate the loss of the least tolerant species (Belmar et al., 2013) and change microbial community composition. Function, structure and composition of aquatic microbial groups living in streambed biofilms and responsible for organic matter cycling, enzymatic activity and ecosystem respiration (Battin et al., 2016), may be critically vulnerable to this process.

Heterotrophic biofilms colonise streambed sediment and during drought they remain temporarily exposed to air and to associate abiotic processes, such as photodegradation and physical disruption (Dieter et al., 2011; Mclaughlin et al., 2017). Although certain bacterial cells can resist desiccation thanks to different resistance strategies (e.g. entering dormancy, maintaining osmotic equilibrium, reducing their growth and respiration rates, or finding refuge in deeper sediment; Orchard & Cook, 1983; Moyano et al., 2013; Sabater et al., 2016), bacteria are generally less tolerant than aquatic fungi which can move more easily across the dry habitat. Aquatic fungi also resist drying better than bacteria because of specific traits such as mycelial growth and spore motility (Manzoni et al., 2014; Mora-Gomez et al., 2016). Nonetheless, although long-term drought may significantly compromise key stream biogeochemical processes at the landscape scale, previous studies observed that streambed microorganisms are able to maintain ecosystem energy flow, extracellular enzyme activities and carbon processing to some extent when the river dries up (Marxsen et al., 2010; Gómez-Gener et al., 2015). The maintenance of microbial function and structure during streambed drought is likely to be linked to the preservation of some water content. As dryness persists, the natural moisture of hyporheic sediment might be essential for heterotroph resistance during long-term drought and could enhance vertical differences in sediment biogeochemical processes (Timoner et al., 2012; Febria et al., 2015; Stegen et al., 2016). Its position between surface and groundwater gives the hyporheic zone the ability to retain water and organic matter (Marmonier et al., 2012; Perujo et al., 2017). For these reasons the hyporheic was defined as an active microbial zone performing crucial processes related to carbon and nutrient cycling where microorganisms can find refuge from harsh conditions (Mermillod-Blondin et al., 2005; Wagner, 2014; Romaní et al., 2017). The occurrence of flash rains may be also critical for the microbiology of dry streambeds, as observed in soils where fast rehydration seems to promote sediment respiration, changes in carbon cycling, and nutrient processing pulses, even after a long dry period (Schimel et al., 2007; Manzoni et al., 2014; Barnard et al.,

2014). The “pulse-reserve” effect observed in desert soils following rain (Noy-Meir, 1973; Reynolds et al., 2004) is expected to occur in the dry beds of temporary waters (Larned et al., 2010; Datry et al., 2017). Flash precipitations during drought periods might alleviate microbial stasis processes, causing activity peaks of carbon degradation (Datry et al., 2014; Evans & Wallenstein, 2014) due to the mineralisation of previously unavailable, easily decomposable organic substrates (Borken & Matzner, 2009), which would facilitate the recovery of the microbial altered biomass and activities, or their persistence (Amalfitano & Fazi, 2008). On the other hand, complete rewetting of the streambed usually causes the recovery of microbial structure and function by for instance promoting nutrient suspension, and cells awaken from the dormant phase (Ylla et al., 2010; Barnard et al., 2013, Aanderud et al., 2015). However, precipitation strength and the frequency of previous drying-rewetting cycles could modulate the lag-time needed to restart microbial growth and biomass recovery as well as determining the amount of cell lysis, as described for soils (Kieft et al., 1987; Blazewicz et al., 2014; Meisner et al., 2015; Székely & Langenheder, 2017).

Knowledge of heterotrophic responses in intermittent streambeds under long-term drought is still limited and unclear. The question of whether a threshold of drought duration could compromise microbial resistance and jeopardise overall resilience needs further insights. Most studies have considered drought durations of between 2 weeks and 2 months and include the measurement of a single set of variables (either focussing on microbial structure or functioning). Here, we considered a five-month drought and key sensitive endpoints such as cell viability and production of extracellular polymeric substances (EPS) together with a range of variables for microbial biomass and heterotrophic functioning in three distinct relevant streambed habitats (surface and hyporheic sediment, and buried leaves).

Our main objective was to study how long-term drought affects heterotrophic microbial structure and function and whether a subsequent rewetting (i.e. water table rise which saturates the surface/subsurface sediment) induces microbial resilience. We use the term “long-term” for our 5-month drought experiment following the NOAA definition that states long-term drought as that lasting for several months to several years, and also mimicking unusual drought duration in Mediterranean intermittent rivers, although in other regions (e.g. Australia) long-term drought could last years. Our, specific objectives were: 1) to measure whether the effects of long-term drought on algal and fungal biomass, bacterial density and viability, content of extracellular polymeric substances and extracellular enzyme activities were distinct depending on the habitat (surface sediment, hyporheic sediment, leaf packs), 2) to investigate the role of flash storms in alleviating long-term drought effects on microbial biomass and enzyme activities, and 3) to describe and quantify the effects of

rewetting in the recovery of microbial biomass and enzyme activities. Two types of substrate (sediment and leaf packs) were considered for the analysis of enzyme activities in order to compare and relate the response of the microbial heterotrophic capabilities in distinct streambed micro-habitats that are relevant for stream organic matter cycling. We hypothesised different microbial responses mainly depending on sediment depth and type of substrate. In particular, for the surface sediment, as being more exposed to drought (drastic decrease in water content), we expected that (i) microbial biomass, activity and viability would drop during the long-term drought, while we supposed that (ii) the damper hyporheic sediment and leaf packs would act as microbial refuges, leading microbial structure and function to be less sensitive to long-term drought. We further expected (iii) that microbial structure and especially function would recover during rewetting and that the flash storms would promote microbial resilience. To address these predictions a long-term experiment, 5-month drought followed by 2-week rewetting, was carried out on 12 sediment columns subjected to three different treatments. From these columns, microbial biomass (fungi, algae, bacterial density and viability), content of extracellular polymeric substances and extracellular enzyme activities linked to plant material degradation (lignin, cellulose, hemicellulose) were measured in surface sediment, hyporheic sediment and leaf packs.

MATERIALS AND METHODS

Experimental design

The long-term drought experiment was performed in 12 streambed sediment columns randomly assigned to three treatments (n=4 replicates per treatment): Control (C, maintained in wet, pool-like conditions), Dry (D, 5 months of drought), and Dry-Storms (DS, 5 months of drought including 2 flash storms). After this five-month drought phase, all treatments were followed by a two-week rewetting phase simulating stream flow recovery and water table resumption (Scheme 1, Fig. S1).

The columns consisted of dark plastic cylinders (25 cm height × 15 cm diameter) with the base perforated with small holes made with a sterile surgical needle (3 mm diameter) to allow water movement through the sediment column. Each column had two little windows (ca. 1 cm^2) at 22 cm depth from the surface and in opposite sides of the column to allow the hyporheic sediment sampling. Also, in one cylinder per treatment, oxygen micro-sensors (sensor spots PreSens®) were positioned at surface and at 22 cm depth inside the column to monitor dissolved oxygen periodically. The 12 columns were prepared in situ in early February 2016 and filled with streambed sediment from the Santa Llúcia de Puigmal stream, a headwater tributary of the Fluvià river (42.217637N, 2.401402E) showing the low summer

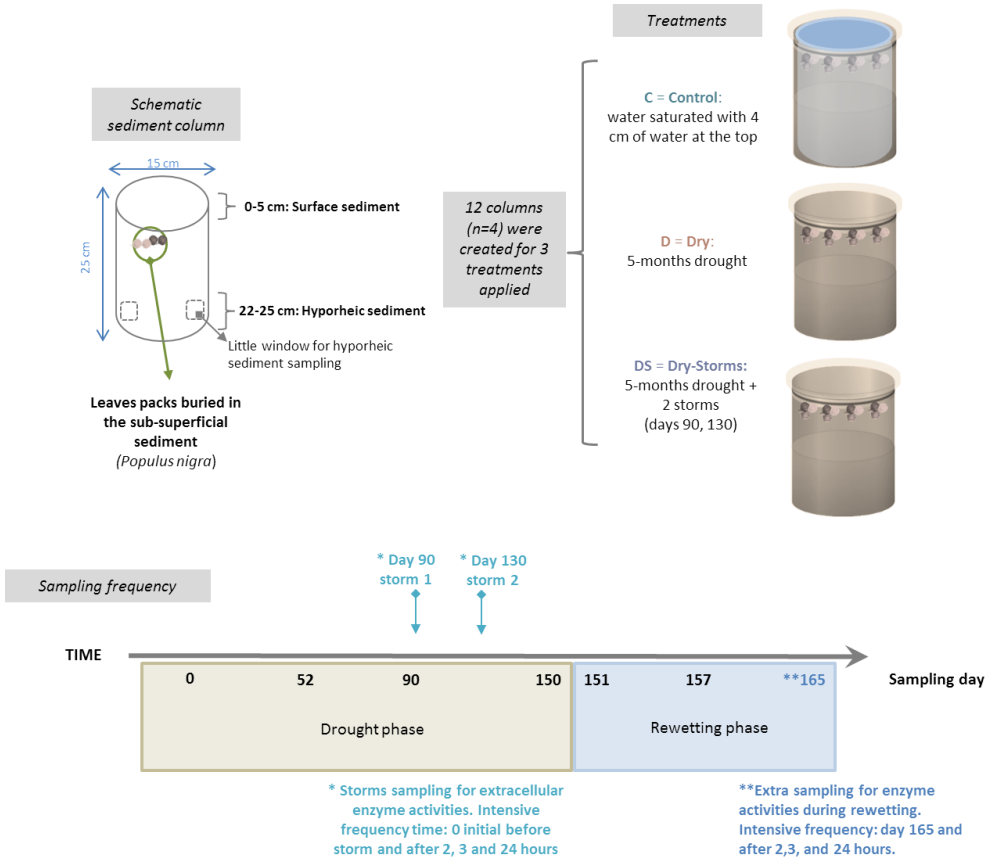
discharge typical of Mediterranean rivers. Water used for the entire experiment (see below) was collected from the same stream site. About 50-75 L of water were collected every two weeks, filtered by 0.2 µm pore size nylon filters (Whatman, Kent, UK) and maintained in fresh conditions (4-10ºC). Before being used, filtered water was left at room temperature for about two hours. Physico-chemical variables in stream water were measured: 0.05 ± 0.12 mg/L N-NO_2, 0.14 ± 0.12 mgL^{-1} N-NO_3, 0.83 ± 0.12 mg L^{-1} P-PO_4 and 10.43 ± 1.38 mg/L of dissolved organic carbon (DOC), water temperature ranged from 7 to 15.6°C, mean dissolved oxygen 10.18 ± 3.58mg/L, mean conductivity 488 ± 41.39 µS/cm and mean pH 8.54 ± 0.06. The physico-chemical variables and nutrients concentrations were further checked with the ACA dataset ("Agència Catalana de l'Aigua" dataset January 2016 - August 2016, Table S1). During the study period the stream was in base flow conditions and no storms occurred.

Sediment columns were filled with homogenised sediment and directly transported to the laboratory. In each column, five leaf litter bags (3x3 cm, mesh size 1 mm) containing 12 leaf circles (12 mm diameter) of *Populus nigra* (collected just after abscission and air dried) were placed in the subsurface sediment layers (at 7-10 cm) and anchored with a thin string to enable bag extraction during sampling. Then each column was placed inside a larger plastic carboy (25 cm diameter, 30 cm depth), filled with fresh filtered river water (3 L to each column) to maintain water saturation in all sediment columns during the first 10 acclimation days before applying the three treatments. Every two or three days, water from all columns was renewed with new filtered stream water to avoid nutrient depletion. Water replacement was performed by emptying the carboys and filling them with new water.

All columns were placed in incubators (SCLAB, PGA 500) under controlled temperature and light conditions. Light conditions for the entire experiment were set at 12h/12h night/day, exposing the columns to a light irradiance (photosynthetic active radiation) of 150 μEm^{-2} s^{-1} (fluorescent lamps) during the day and darkness during the night. The range of temperatures applied during the experiment was planned according to average values during the spring-summer period recorded in the river where sediment and water were collected (data 2005-2015 Idescat). For the first 10 days, the columns were subjected to a diel cycle of mean spring temperature (16ºC/20ºC, night/day). After the first 10 days of acclimation, temperature was increased 1ºC every week during 42 days (reaching 25-27ºC), the following 15 days temperature was maintained at 27-29ºC simulating late spring and summer temperatures and the last 84 days of the long-term drought and 15 days of rewetting the temperature was maintained at 30-31ºC (summer conditions). Night-time temperature was 4 degrees below the daily value. Temperature fluctuations within the twelve positions inside the incubators had been tested previously with temperature data loggers (ACR SmartButton Logger, MicroDAQ)

which showed some variability between positions (error ± 0.5ºC). This effect was minimised by applying a rotation positions protocol of the sediment columns throughout the experiment.

After conditioning the columns, which was necessary to avoid artefacts in the analysis due to sediment settlement, the 3 treatments were imposed. For the control treatment (C) columns were maintained in saturated conditions with 4 cm of a water layer at the top of each column and water was renewed twice a week. For the dry treatment (D) columns were left to dry, external carboys were emptied and no extra water was added for the next 150 days. For the dry-storms (DS) treatment columns were also left to dry as in D treatment, but at days 90 and 130, 750 mL of stream diluted water (1:1, filtered stream water : distilled water) were added during 30 min (simulating summer storms in that area, dataset 2005-2015 ACA – "Agència Catalana de l'Aigua"). At day 150, all sediment columns were rewetted and maintained under saturated conditions with filtered stream water renewed every two days. Rewetting was performed by filling the outside carboys in order to simulate water table rise with the percolation of water up from the bottom of the columns and, at the same time, part of the water was poured into top of the columns to simulate precipitation and allow the complete wetting of the whole sediment column.



Scheme 1. The complete structure of the experimental design.

In the context of this study we used the terms surface and hyporheic sediment for the portion of sediment between 0 to 5 cm depth and from 22 to 25 cm depth, respectively. Surface sediment from each column was collected from a different position each time, following a circle to avoid re-sampling the same place twice. Surface sediment was sampled using a small syringe (2 cm diameter, 1x100 NORM-JECT®, Henke Sass Wolf Germany) for a total of 10-12 cm^3. The same amount of hyporheic sediment was collected by opening one of the two little windows we created before filling the sediment containers. At each sampling time, hyporheic sediment (10-12 cm^3) was collected with a thin spatula and then the window was closed and hermetically sealed. Sampling alternates between the two windows and, although sampling made a little hole in the column, the time between sampling allowed the sediment to settle again. Once assembled, each sample was split into subsamples of 0.5mL (organic matter; extracellular enzyme activities) or 1mL (algal and fungal biomass; bacterial density; bacterial viability; extracellular polymeric substances) and distributed using a smaller syringe (1 cm diameter, 1x100 NORM-JECT®, Henke Sass Wolf, Germany) while the remaining sediment (about 4 mL) was used for sediment characterization.

One leaf bag from each sediment column was extracted at days 0, 52 and 150 during the drought phase, and after 24h and 7 days from rewetting. Surface (0-5 cm) and hyporheic (20-25 cm) sediment was sampled from each replicate at days 0, 52, 90 and 150 during the drought phase, and after 24h and 7 days from rewetting during the rewetting phase. Sediment sampling frequency for extracellular enzyme activities followed a more intense and longer monitoring during the rewetting phase (after 1, 3, 24 and 72 hours and after 7 and 15 days from rewetting) to obtain a more detailed functional response. Furthermore, extracellular enzyme activities in surface and hyporheic sediment in the DS treatment were also measured intensively during the storm events: just before each storm (time 0), and after 1, 3, and 24 hours from each event. Sediment samples for EPS (extracellular polymeric substances) and fungal biomass were stored frozen at -20ºC, samples for chlorophyll-a were directly filled with acetone for chlorophyll extraction and analysed the following day, and samples for bacterial density were stored at room temperature with 10 mL of filtered stream water and formaldehyde (2%). Sediment samples for bacterial viability were analysed the same day as sampling. For leaf litter sampling, leaf discs were carefully rinsed with distilled water to eliminate sediment particles and four discs were separated to measure extracellular enzyme activities while the remaining eight were used for dry weight (DW) measurements. Extracellular enzyme activities in sediment and leaves were measured immediately after sampling. Extra sediment for sediment characteristics analysis was kept at 4ºC until analysis.

Organic matter, oxygen, water content and sediment characteristics

Organic matter (OM) content was measured as AFDW (Ash Free Dry Weight) for each 0.5 mL of sediment sample which was dried at 70°C during 72 h and burnt for 4 h at 450 °C using a muffle furnace (AAF 1100, Carbolite, UK). The results were expressed as grams of AFDW per gram of sediment DW. Water content was calculated as percentage of water loss (%) obtained by the difference between fresh and dry weight. The content of dissolved oxygen was measured nine times at each sediment depth and treatment throughout the experiment. To determine the grain size distribution, fresh sediment samples were first treated with H_2O_2 (10% volume) to remove organic matter and later disaggregated and dispersed ultrasonically with pyrophosphate. Fractions up to 2 mm were determined by sieving, while determination of fractions below 2 mm was performed with a Beckman-Coulter LS230 laser.

Microbial biomass and content of extracellular polymeric substances (EPS)

Chlorophyll content.

Chlorophyll-a (Chl-a) extraction from sediment samples was accomplished as described by Jeffrey and Humphrey (1975). Acetone 90% was added to 1 mL of sediment for 12 h in the dark at 4°C. To ensure complete extraction of pigments, samples were further sonicated for 2 min (sonication bath Selecta S.A.). Chl-a concentration of filtered extracts (fibreglass GF/C, Whatman) was determined in a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) measuring absorbance at 430 nm, 665 nm and 750 nm wavelengths. Results were expressed as µg of Chl-a per g of sediment DW.

Bacterial density and viability.

Bacterial cells were detached from sediment after 60s of ice-sonication (Selecta, 40W and 40 kHz) and then vortex. One mL subsample was purified using the Nycodenz density gradient centrifugation (Optiprep, density gradient medium), following the description by Amalfitano & Fazi (2008), in order to clarify the sample by separating the cells from the sediment particles. Sample preparation and cytometry analysis followed the procedures described in Perujo et al. (2017). Results are given as cells × 10^6 per grams of sediment DW. Bacterial viability was estimated by flow-cytometry using Live/Dead bacterial viability kit (Invitrogen Molecular Probes, Inc.). One mL of sediment was collected from each depth and placed in a sterile vial with 5 mL of Pyrophosphate solution 50 mM (Manini & Danovaro, 2006) and following the procedures described in Perujo et al. (2017). Results are given in % of live cells.

Fungal biomass.

Fungal biomass in sediment samples was estimated from ergosterol content. Preserved frozen samples were lyophilised before the analysis (Laboratory Freeze Dryer

CRYODOS, Telstar industrial S.L., Spain). Ergosterol was extracted using KOH methanol 0.14 M at 80 ºC for 30 min, and then separated by solid-phase extraction (Gessner & Schmitt, 1996). Ergosterol was detected and quantified using high pressure liquid chromatography (HPLC; Waters Inc., Milford, MA, U.S.A.) at 282 nm. Quantification was based on the comparison with ergosterol standards (0 – 200 µg mL-1, Fluka Chemical Co., Steinheim, Germany). Fungal biomass in terms of fungal carbon content was estimated based on an ergosterol content of 5.5 mg g-1 fungal biomass (Gessner & Chauvet, 1994) and 43% carbon content in fungal dry mass (Baldy & Gessner, 1997). Results are expressed as grams of fungal carbon per gram of sediment DW.

Polysaccharide content in Extracellular Polymeric Substances (EPS).

Sediment samples for polysaccharide content in EPS were frozen and preserved at -20ºC prior to analysis. Extracellular polymeric substances were extracted from the sediments using cation exchange resin (CER, Dowex Marathon C sodium form, Sigma-Aldrich) and following the procedure described in Romaní et al. (2008). Results are given as glucose-equivalents per gram of sediment DW.

Extracellular enzyme activities (EEA)

The extracellular potential enzyme activities of β-D-glucosidase (EC 3.2.1.21, GLU), β-D-xylosidase (EC 3.2.1.37, XYL) and phenol oxidase (EC 1.14.18.1, PHE) were measured in sediment and leaf litter disc samples as indicators of the capacity to decompose cellulose, hemicellulose and lignin compounds, respectively.

Briefly, sediment samples (0.5 mL) and leaf litter discs (1 leaf circle) were placed in 15 mL falcon tubes with 4mL of filtered stream water (0.2 µm pore size, nylon, Whatman). For each hydrolytic enzyme (GLU and XYL), blanks without sample and a standard curve of MUF (methylumbelliferone, Sigma-Aldrich) were also incubated. In each sample 120µL of MUF artificial substrate (MUF-β-D-glucoside and MUF-β-D-xylopyranoside; Sigma-Aldrich, final concentration of 0.3 mM) were added and samples were incubated under continuous shaking (150 rpm) for 0.5h in dark conditions at 20ºC. At the end of the incubation, 4 mL of glycine buffer (pH 10.4) were added to each vial to stop the reaction and maximise MUF fluorescence. Samples were centrifuged (2000g) for 2 min, and the supernatant for each sample (340 µL) was placed into 96 well black microplates (Cell grade, Brand plates) for fluorescence measurements. The fluorescence was measured at 365/455nm excitation/emission in a fluorimeter plate reader (Tecan, infinite M200 Pro) with a bandwidth of 5 nm. Values were expressed as nmol MUF gDW-1 h-1.

PHE activity was assayed using L-3,4 dihydroxyphenylalanine (L-DOPA) at a final concentration of 1.5 mM with acetate buffer (pH 5). Each sample (0.5 mL of sediment or 1 leaf

circle) was placed in vials filled with 2 mL of filtered stream water (0.2 µm pore size, nylon, Whatman) and 2 mL of L-DOPA solution. Control samples with acetate buffer (without L-DOPA) were also incubated. The incubation lasted 0.5 h under continuous shaking (150 rpm) in dark conditions at 20ºC. Blanks with acetate buffer and L-DOPA solution were also incubated. Samples were centrifuged (2000g) for 2 min, and the supernatant for each sample (340 µL) was placed into 96 well transparent microplates (MicroWell™ Nunc™, ThermoFisher Scientific) for absorbance measurements at 460 nm using a microplate reader (Tecan, infinite M200 Pro). The values were expressed as nmol 2,3-dihydroindole-5,6-quinone-2-carboxilate (DIQC) gDW-1 h-1. For the entire study period, the recalcitrant index was calculated as the ratio of log glucosidase activity and log phenol oxidase activity, as previously described by Sinsabaugh (2010).

Leaf litter decomposition

Leaf litter discs in each bag were dried (70 ºC, 72 h) and weighed. Decomposition rates were estimated for comparative purposes by linear regressions of log-transformed data of percentage of the DW remaining (negative exponential model Mt = M0*e-kt, where M0 is the initial DW, Mt is the remaining DW at time t, and k is the decomposition rate).

Data analysis

Linear mixed models (LMM) for repeated measures were applied to analyse how the long-term drought and rewetting phases affect microbial structure and function. For each LMM and response variable we selected the best covariance structure based on Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC). All the models performed presented scaled identity or diagonal as the best covariance structure. The experimental phases (drought and rewetting) were studied separately with depth (DE), treatment (TR) and time (TI) as fixed effects factors and the sediment columns as a random factor. The comparison between the effects driven by flash storms and rewetting on EEA were studied through LMM with storms (ST, which included two storms and final rewetting), time (TI, repeated measures) and depth (DE) as fixed factors. When interactions between TR and DE were significant, independent analysis for surface and hyporheic sediments were performed to highlight the treatment effects (pairwise comparisons with Sidak test). The relationship between sediment moisture and the structural and functional variables most affected (bacterial viability, EPS, GLU and XYL activities) was tested with LMM for repeated measures including water content as covariate. Separate models for surface and hyporheic sediment were performed and only D and DS were introduced in this analysis. The structural and functional resilience was calculated as the percentage of difference between the control (C) and the values measured at the dried treatments (D and DS) at the end of the rewetting. Previous to this calculation we ensured that

at time 0 (initial) there were not significant differences between treatments and then we tested the presence of significant differences between treatments at the end of the rewetting (one-way ANOVA with Dunnet's test pairwise comparison). When no significant differences between D/DS and C were found we considered this specific variable to be resilient to the long-term drought. The relationships between structural and functional variables were assessed by correlation analyses, across the whole experiment. All of these statistical analyses were performed using the IBM SPSS STATISTICS 23 software (SPSS Inc., USA). Apart from bacterial viability and water content, all response variables (y) were log(y) or square-root(y) transformed, to fulfil normality and homoscedasticity assumptions. Finally, analysis of covariance (2-way ANCOVA, test for homogeneity of slopes) was used to compare the treatment effects on leaf litter decomposition rate (log of %DW remaining as the dependent variable), using time as a covariate. Calculations were made with the CSS Statistical package.

OUTCOMES

During the drought phase (Table 1), the trends through time for almost all measured variables were affected by treatment and this effect was different depending on the depth (TR×TI×DE effects, Table 1). During the rewetting phase, this 3-way interaction was significant for water content, bacterial viability, XYL and PHE activities (Table 1). Due to this significance of the triple interaction and in order to distinguish the treatment effect, the statistical results from separate analyses (LMM) divided per sediment depth and per different experimental phase are shown in each figure.

Table 1. p-Values from linear mixed models (LMM). For each experimental period (Long term drought; Rewetting) we tested a full factorial model with three fixed effects factors: treatment (TR), depth (DE) and time (TI), the last as the repeated measures factor.

	Source of variation	Water	OM	Chl-a	Bacteria	Live cells	Fungi	EPS	GLU	XYL	PHE	GLU:PHE
		p value										
Long term drought	*TR*	**<0.001**	0.354	**<0.001**	0.071	**0.001**	**<0.001**	**0.008**	**0.001**	0.164	**0.017**	**<0.001**
	TI	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	DE	**<0.001**	**0.003**	**0.031**	**<0.001**	**<0.001**	0.188	**0.002**	**<0.001**	**0.012**	0.779	**<0.001**
	TR x TI	**<0.001**	0.272	**<0.001**	0.901	**<0.001**	**<0.001**	0.179	**0.001**	0.258	0.732	0.240
	TR x DE	**<0.001**	**0.001**	**<0.001**	0.443	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**0.001**	**<0.001**
	TI x DE	**<0.001**	0.403	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.006**	**0.001**	**<0.001**
	TRxTIxDE	**0.001**	**0.027**	**<0.001**	0.083	**<0.001**	**0.002**	**0.007**	**<0.001**	**<0.001**	0.518	**<0.001**
Rewetting	*TR*	**0.002**	0.481	**<0.001**	0.164	**<0.001**	0.655	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	TI	**<0.001**	0.648	0.166	**<0.001**	**<0.001**	0.239	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	DE	**<0.001**	0.923	**<0.001**	**0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**0.027**	**<0.001**
	TR x TI	0.148	**0.003**	**0.045**	0.208	0.346	0.940	0.735	**<0.001**	0.676	0.194	0.431
	TR x DE	0.435	0.687	**<0.001**	0.626	**0.001**	0.502	**0.009**	0.065	**<0.001**	**0.001**	**<0.001**
	TI x DE	0.211	0.343	0.092	**0.001**	**<0.001**	0.314	0.640	**<0.001**	**<0.001**	**<0.001**	**0.048**
	TRxTIxDE	**0.023**	0.823	0.165	0.129	0.029	0.570	0.773	0.410	**<0.001**	**0.045**	0.837

Response variables: Water, water content; OM, organic matter; Chl-a, chlorophyll-a; Bacteria, bacterial biomass; Live cells, bacterial viability; Fungi, fungal biomass; EPS, extracellular polymeric substances; GLU, β-glucosidase; XYL; β-xylosidase; PHE phenol oxidase. Significant p-values (<0.05) are indicated in boldface. The p-values of extracellular enzymes during rewetting period are referred to the longer time series considered for the enzyme activities (until day 165).

Water content, organic matter, oxygen conditions and sediment characteristics

During the drought phase (except for day 0), water content in both sediment depths was significantly higher in C than in D and DS treatments and it significantly increased at day 150 in DS hyporheic sediment (Fig. 1a). During the drought phase water content in surface sediment was on average 29.08%, 0.54% and 1.41% for C, D, and DS treatments, while the average in hyporheic sediment was 27.28%, 2.51% and 4.32% in C, D and DS treatments, respectively. The rewetting restored the sediment water content in D and DS to the same values as in the C treatment. This recovery was faster in the hyporheic (day 151) than in the surface sediment (day 157, Fig.1).

No significant differences in OM content were observed between treatments during the drought phase in surface sediment, which ranged between 0.009±0.001 and 0.012±0.002 gAFDW/gDW (Fig. S2). At the end of the drought phase (day 150), OM in hyporheic sediment rose significantly in DS than in C (Fig. S2) while during the wet phase, C treatment doubled its OM content during the first 24 hours (day 151), showing significantly higher values than those

in D and DS (Fig. S2). After 7 days of rewetting (day 157), OM content was similar in both sediment depths, 0.010±0.001 gAFDW/gDW, without differences between treatments (Fig. S2).

The average concentration of dissolved oxygen (DO) during the drought phase in C treatment was 4.07±1.78 mg/L at the surface and 0.07±0.04 mg/L at the bottom of the columns. Conversely, the mean DO concentration in D and DS treatments was 8.54±0.49 and 8.71±0.58 mg/L at the surface, and 8.50±0.65 and 8.31±1.54 in the hyporheic sediment. Once rewetted, DO concentration ranged between 5 - 7 mg/L for all treatments and both sediment depths.

In terms of grain size characterization, sediments were dominated by coarse fractions: sand and coarse-sand. The finest fractions (i.e. silt and clay) were always below 10% and during long-term drought, but slightly higher in hyporheic sediment (Table S2). Silt + clay percentage decreased with time in a similar way in the three treatments and in surface and hyporheic zones, during drying period (Table S2).

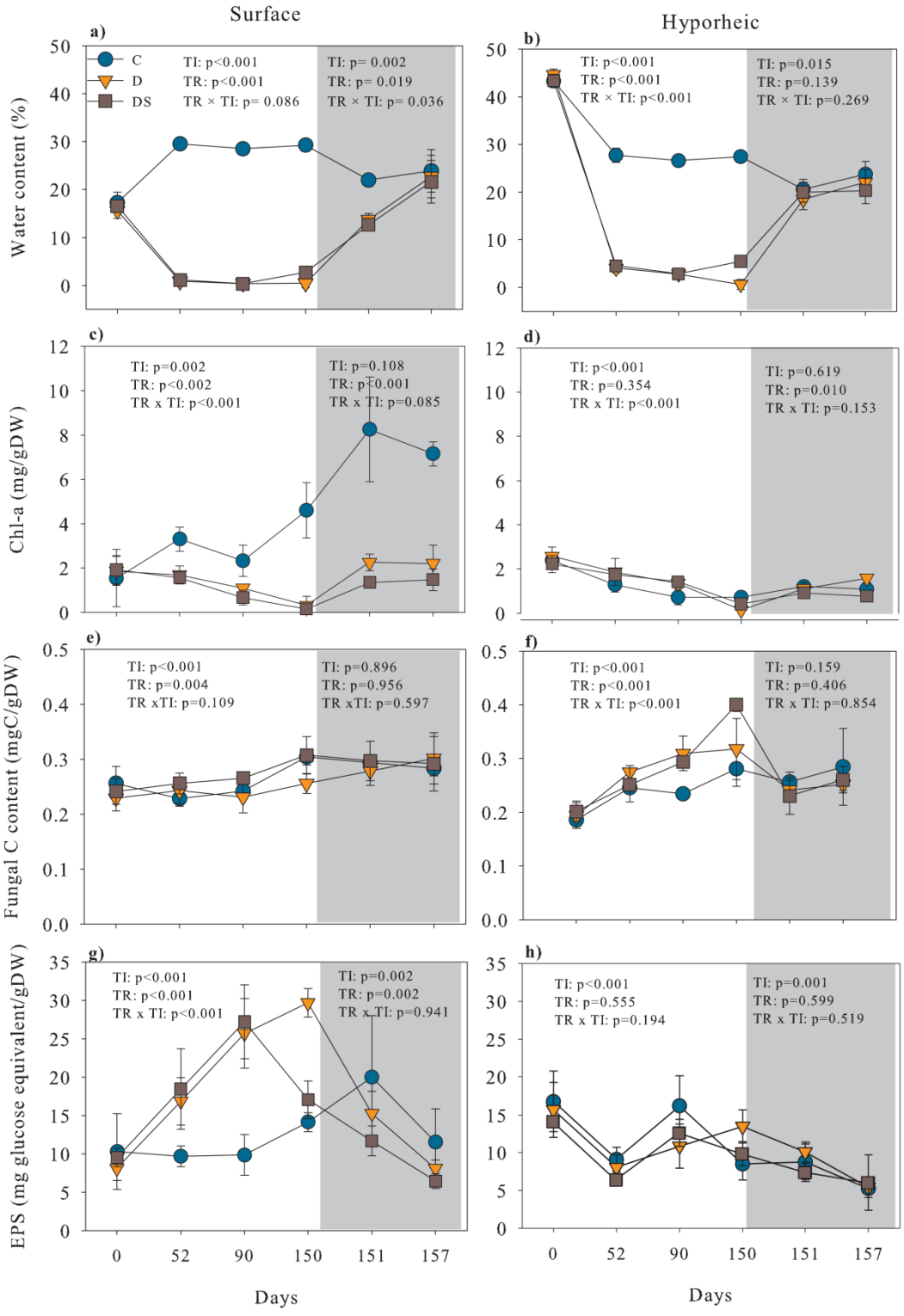


Figure 1. In surface (left column) and hyporheic (right column) sediment:(a-b) Percentage of water content; (c-d) Chlorophyll-a content (µg/gDW); (e-f) Fungal carbon content (mgC/gDW); (g-h) Extracellular polymeric substances content (EPS in µg glucose-equivalents/gDW) for the three treatments (C: Control, D: Dry, DS: Dry-Storms) during the long-term drought and rewetting (shaded area) experimental phases. At day 90 and 130 two storms occurred. Values are mean ± SD (n=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

Chlorophyll, fungi, bacteria, and EPS content

Chlorophyll content

During the drought phase, Chl-a content in surface sediments slightly decreased in D and DS treatments, while it significantly increased in the C treatment (Fig. 1c). In the hyporheos, differences between treatments were less evident, but the temporal trends were still significantly different between treatments and D and DS showed a greater decrease from days 90 to 150 than the C treatment (Fig. 1d).

In the rewetting, Chl-a increased for all treatments during the first 24 hours (day 151), especially in surface sediments, where differences between treatments were maintained as for the drought phase with significantly higher values in C than in D and DS treatments (Fig. 1c). At the end of the rewetting, Chl-a in surface sediment was 4 times the initial experimental value for the C treatment, while for the D and DS, values were similar than the initial ones. In the hyporheos and in the rewetting, differences between treatments were much less evident (only slightly higher Chl-a content in D than in DS treatment) and values were lower than the initial ones (Fig. 1d).

Fungal biomass

During the drought phase, fungal biomass in surface sediment was maintained quite constant with a greater increase in DS and C than in D treatment at day 150 (significant difference between DS and D, Fig. 1e). In hyporheic sediment, fungal biomass content rose significantly in D and DS until day 90, and at day 150 in DS treatment, after the storm episodes, peaked at the maximum value, becoming separated from C and D (Fig. 1f). The rewetting caused a decrease in fungal biomass in the hyporheic sediment which resulted in higher fungal biomass in the surface than in the hyporheic, but no differences between treatments were observed (Table 1, Fig. 1e, f). Once the wet phase ended, all the treatments showed higher values than the initial ones (Fig. 1e, f).

Bacterial density and viability

Bacterial density was almost not affected by the drought treatments (excepting slightly higher values in the C treatment in surface sediments during drought) and showed a tendency of decreasing during the experiment both in surface and hyporheic sediments (Fig. 2a, b). In contrast, bacterial viability was significantly affected by the drought treatments. During the drought phase, bacterial viability in surface sediments was higher in C than in D and DS treatments and after the storm episodes it increased in the DS treatment to values equal to C until the end of the experiment (Fig. 2c). In the hyporheos, bacterial viability was higher than in surface and differences between treatments were especially evident at day 150 (after the storm episodes) when higher values were found for DS than D and C treatments (Fig. 2d). After

7 days of rewetting, all the treatments showed higher than initial values in surface sediments and DS bacterial viability rose to values significantly higher than D and C both in surface and hyporheic zones (Fig. 2c, d).

Extracellular polymeric substances, EPS

The EPS content, throughout the drought phase, was increasing significantly in D and DS treatments in the surface sediment, while it was not showing significant differences between treatments at the hyporheic sediment (Fig. 1g, h). After the storm episodes (day 150), EPS content in surface DS sediment decreased to values similar to those in the C treatment (Fig. 1g). In the rewetting phase, the differences between treatments in surface sediment were reduced, as EPS content decreased in D and DS after 24 hours of rewetting (Fig. 1g). At the end of the rewetting phase (day 157) EPS content of both sediment depths decreased in all treatments, reaching values equal to or below the initial ones in the case of hyporheic sediment (Fig. 1h).

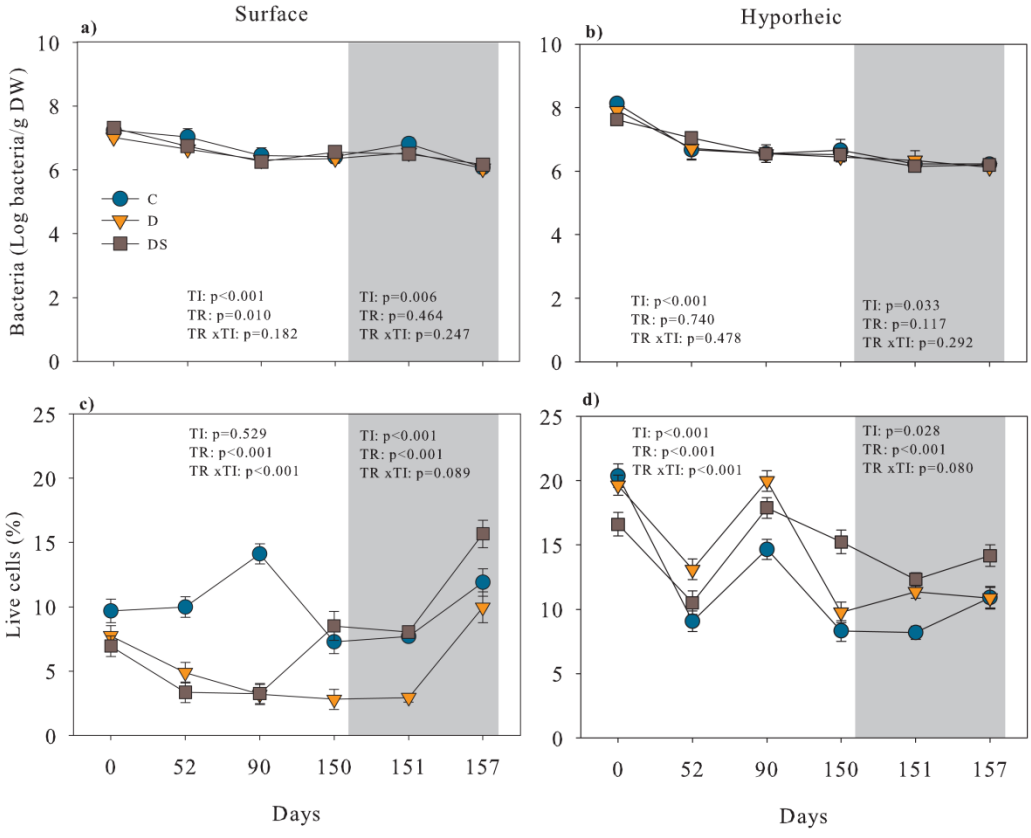


Figure 2. In surface (left column) and hyporheic (right column) sediment: (a-b) Bacterial density (log Bacteria cells/gDW); (c-d) bacterial viability (Live cells %), for the three treatments (C: Control, D: Dry, DS: Dry-Storm) during the long-term drought and rewetting (shaded area) experimental phases. At day 90 and 130 two storms occurred. Values are mean ± SD (n=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

Extracellular enzyme activities in sediment and leaves, and during storm events

During the drought phase, GLU and XYL activities in surface sediments were significantly higher in C than in D and DS treatments (Table 1, Fig. 3a, c). PHE activity in surface sediments also showed slightly higher values in C treatment and drastic time fluctuations (Fig. 3e). In contrast, in the hyporheic sediments the treatment effect was opposite to that observed in the surface and the three enzyme activities showed lower values in C than in D and DS treatments (Fig. 3b, d and f). This was especially significant for GLU and XYL, where significantly higher values were measured in D and DS than in C (Fig. 3b, d).

After one hour of rewetting, differences in surface GLU activity between treatments were still significant, but values in DS doubled, becoming separated from those in D until the end of the rewetting phase (Fig. 3a). This occurred similarly but more drastically for XYL and values in DS rose about 3 times and reached the same values as those in C treatment at one hour after rewetting (Fig. 3c). PHE activity in surface sediments depicted an increasing trend during the rewetting phase with high time variability and no significant differences between treatments (Fig. 3e). In the hyporheic sediment, GLU and XYL still maintained lower values in C treatment and showed the same trends trough time for all treatments (Fig. 3b, d). On the other hand, the temporal trend of PHE activity at the hyporheic layers was different between treatments and after 2 weeks of rewetting (day 165) DS rose significantly (Fig. 3f), increasing to almost 4 times its value compared to the one at the end of the drought phase (day 150). After 2 weeks of rewetting (day 165), PHE activity showed final values higher than the initial ones in all treatments and at both sediment depths (Fig. 3e, f).

The recalcitrance index (GLU:PHE) showed higher values in C than in D and DS treatments in surface sediment throughout the experiment (Table 1, Fig. 3g). In contrast, in the hyporheic sediment, no significant differences between treatments in the recalcitrance index were found (Fig. 3h).

GLU and XYL activities measured in leaf litter showed differences between treatments both during the drought and rewetting phases. For GLU, higher activities were measured in DS treatment and for XYL higher activities were measured for both C and DS treatments (Table 1, Fig. S3). At the end of the rewetting lowest activities were measured in the D treatment (Fig. S3). No effect of treatment was detected for PHE activity and the recalcitrant index (Table 1, Fig. S3).

The exposure to two storm events in the DS treatment showed similar patterns for all the enzymes during the first 24 hours, showing in general higher and steadier values in the hyporheic sediment while enhanced time variability was observed at the surface sediment (Fig. S4). Temporal trends of enzymes were affected by the storm event and this effect was

different depending on the depth (ST × TI × DE, Table S3). At the surface the three enzymes showed a positive highpoint after one hour of the first storm (ST1), which was significantly higher in the case of GLU and XYL (Table S4, Fig. S4a,c). The same higher and significant peaks were observed for all the surface activities (Fig. S4a,c,e) after one hour of rewetting (RW) as well as for the hyporheic PHE (Table S4, Fig. S4f). Conversely, the second storm (ST2) did not respond with significant changes for all the activities through time and for both sediment depths (Fig. S4).

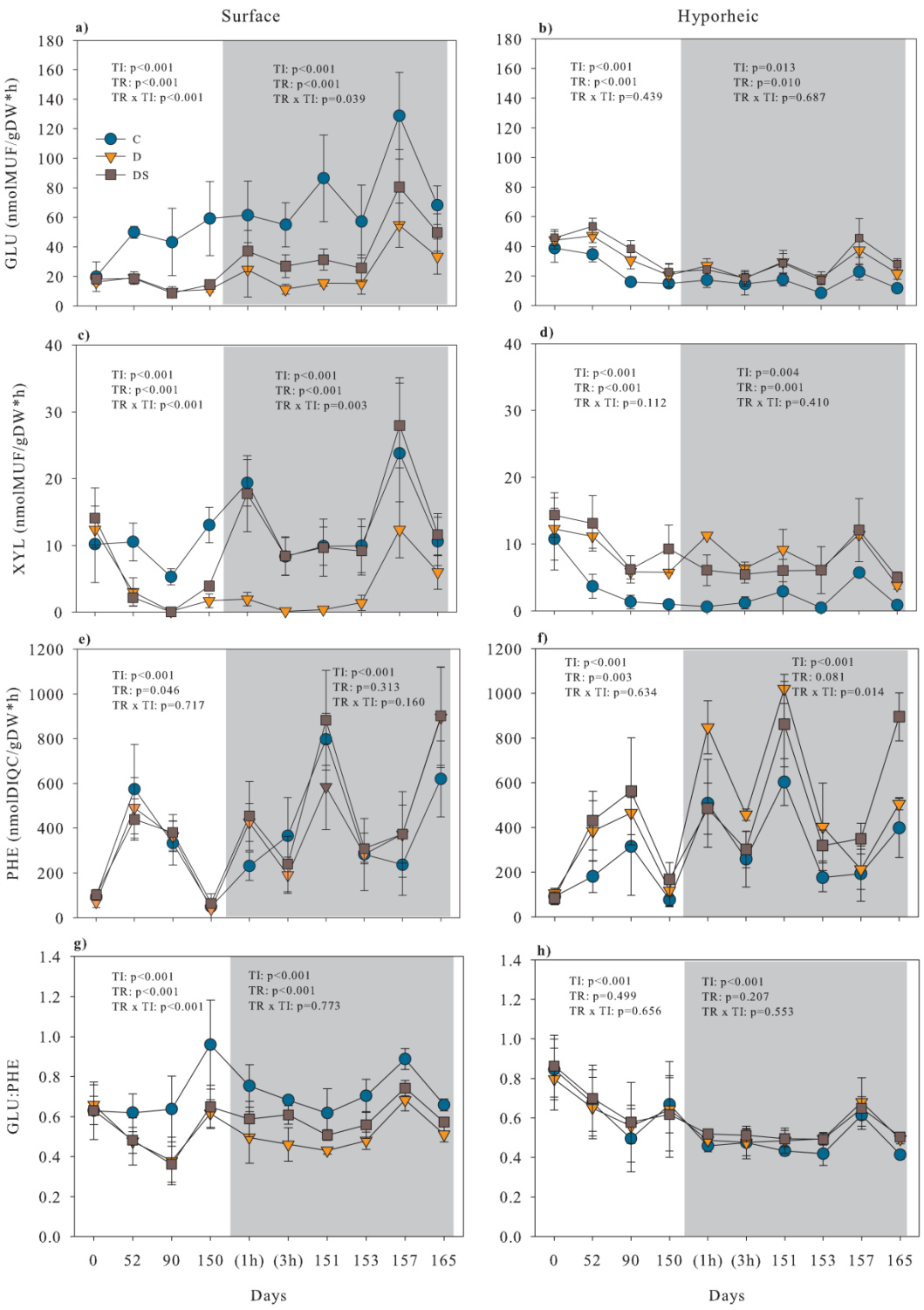


Figure 3. In surface (left column) and hyporheic (right column) sediment: (a-b) β-D-glucosidase (GLU); (c-d) β-D-xylosidase (XYL); (e-f) phenol-oxidase (PHE); (g-h) Recalcitrant index (GLU:PHE) for the three treatments (C: Control, D: Dry, DS: Dry-Storms) during the long-term drought and rewetting (shaded area) experimental phases. At day 90 and 130 two storms occurred. Values are mean ± SD (n=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

The EEAs showed several significant correlations to the structural variables (Table 3). GLU and XYL correlated positively to bacterial viability and XYL further correlated to bacterial biomass. In contrast, PHE was negatively correlated with bacterial and fungal biomass. The three EEAs correlated positively with chlorophyll-a and negatively with EPS content.

Table 3. Correlations coefficients (Corr.) and significance (Sign.) between structural and functional variables studied. The whole experimental time, treatments and both sediment substrata have been considered.

	Live cells		**EPS**		**Chl-a**		**Bacteria**		**Fungi**	
	Corr..	Sign.	Corr..	Sign.	Corr..	Sign.	Corr..	Sign.	Corr..	Sign.
GLU	*0.374*	**0.0001**	*-0.202*	**0.015**	*0.611*	**0.0001**	*0.111*	0.187	*-0.127*	0.129
XYL	*0.331*	**0.0001**	*-0.309*	**0.0001**	*0.343*	**0.0001**	*0.175*	**0.036**	*0.033*	0.695
PHE	*0.151*	0.071	*-0.187*	**0.025**	*0.288*	**0.0001**	*-0.214*	**0.01**	*-0.292*	**0.0001**

Structural variables analyzed: Live cells, bacterial viability; EPS, extracellular polymeric substances; Chl-a, chlorophyll-a; Bacteria, bacterial biomass; Fungi, fungal biomass, in relation with the three functional variables of extracellular enzymatic activities (GLU, β-glucosidase; XYL, β-xylosidase; PHE, phenol oxidase). Significant p-values are indicated in boldface.

Leaf decomposition

Decomposition processes differed significantly between treatments during the study period (ANCOVA, $F_{2,68}=10.2$, $p<0.0001$; Fig. S5), with higher decay rates measured in C and DS treatments (0.0030 and 0.0029 day-1, respectively) than in D treatment (0.0015 day-1, $p<0.005$ Tukey test). At the end of the drought phase and after the storm events in DS treatment (day 150), the percentage of DW remaining was significantly lower in DS (46.094 ± 3.254%) and C (54.143 ± 5.023 %) than in D (60.523 ± 2.415 %) treatment (Fig. S5). Seven days after rewetting (day 157), the weight loss continued to be significantly pronounced in DS and C compared to D (Fig. S5).

Resilience of microbial structure-function variables and relationship with water content

Structure and function variables at the beginning of the experiment did not show differences between treatments ($p>0.05$), then differences at the end of the rewetting were calculated (Table S5) and were significant for Chlorophyll-a, EPS, live bacteria, GLU, XYL and PHE. At the end of rewetting, Chl-a content in surface sediment was significantly lower in D and DS than in the C (about 80% of reduction), while in the hyporheic no differences between treatments were measured (Fig. 4, Table S5). EPS in the surface DS treatment showed a value

50% lower than in C (Fig. 4, Table S5) whereas bacterial viability was higher in DS than in C both in surface and hyporheic sediment (Fig. 4). Activities showed an opposite pattern in surface and hyporheic sediment, in surface GLU activity in D was lower than in C, while in the hyporheic, GLU and XYL were higher in D and DS than in C, and also for PHE in DS (Fig. 4, Table S5).

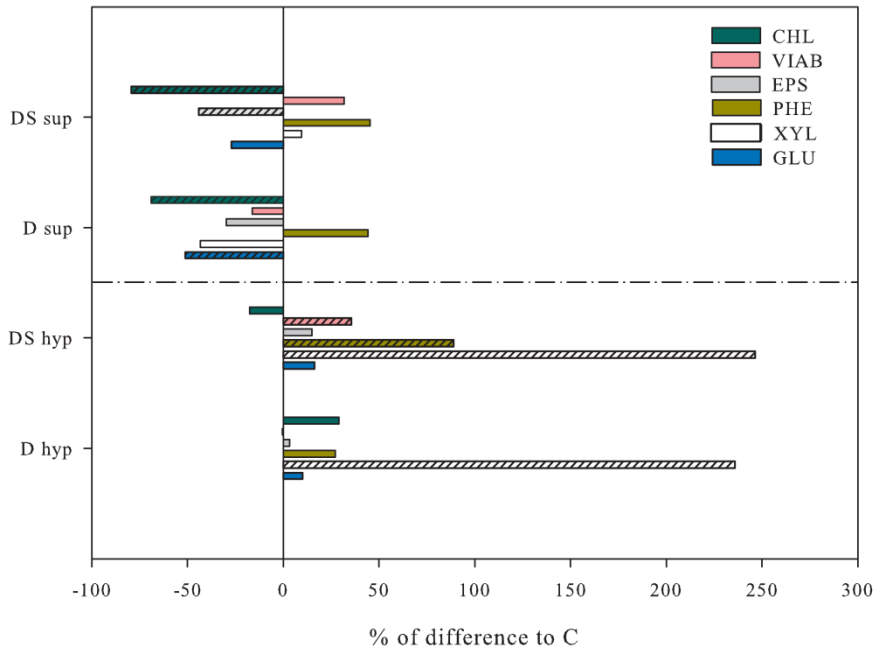


Figure 4. Percentage of difference at the last day of rewetting period (day 157 or day 165 in the case of extracellular enzyme activities), between dry treatments (D and DS) and control treatment (C), at surface (sup) and hyporheic (hyp) depth for β-Glucosidase (GLU), β-Xylosidase (XYL), Phenol-oxidase (PHE), Chlorophyll-a (CHL), bacterial viability (VIAB), Extracellular polymeric substances (EPS). Line pattern indicates significant differences obtained from pairwise comparisons performed between C and D, and C and DS (ANOVA analysis with Dunnet's post hoc test).

Significant relationships between sediment water content and bacterial viability, EPS content, GLU and XYL activities were found in the surface sediment for D and DS treatments during the long-term drought (Fig. 5, Table 2). In the hyporheic sediment, only slight significant relationships were found between sediment water content and EPS and XYL variables (Fig. 5, Table 2) while no interaction between treatment and water content was found in any sediment compartment (Table 2).

Table 2. p-Values from linear mixed models (LMM) for each depth compartment (Surface and Hyporheos), considering water content (WC) as covariate. Only dry (D) and dry-storm (DS) treatments have been considered.

Source of variation	Live cells	EPS	GLU	XYL
Surface		*p values*		
TR	0.706	0.222	0.845	0.983
WC	**<0.001**	**<0.001**	0.053	**<0.001**
WC x TR	0.372	0.100	0.827	0.893
Hyporheic				
TR	0.617	0.329	0.265	0.735
WC	0.118	**0.025**	0.387	**0.016**
WC x TR	0.262	0.728	0.554	0.518

Response variables: Live cells, bacterial viability; EPS, extracellular polymeric substances; GLU, β-glucosidase; XYL, β-xylosidase; PHE, phenol oxidase. Significant p-values (<0.05) are indicated in boldface.

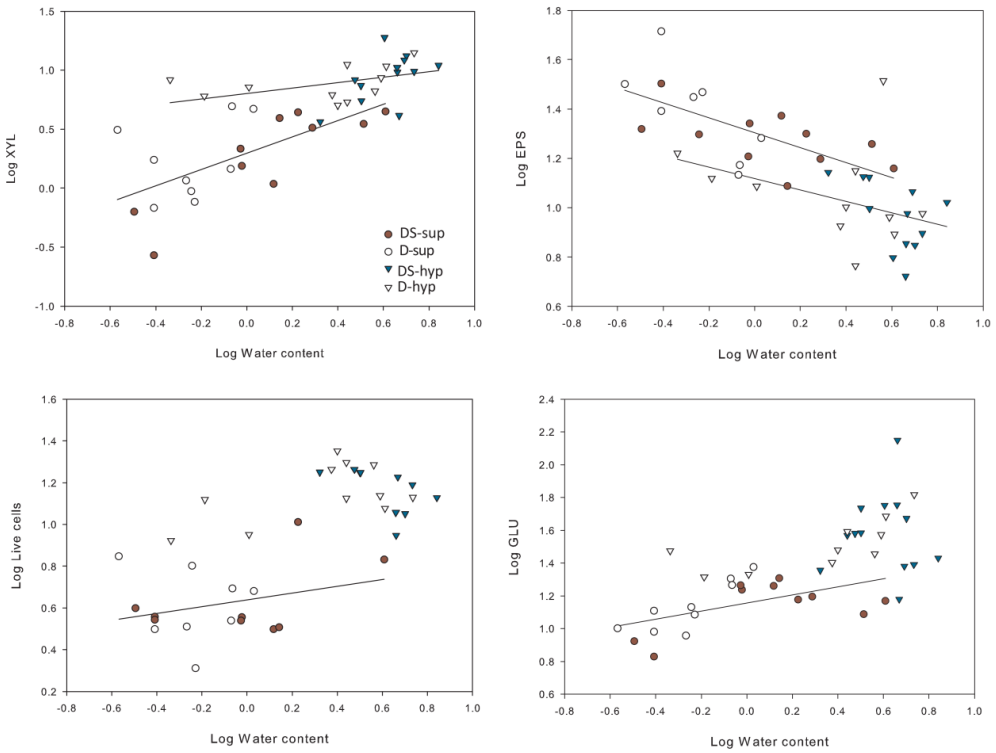


Figure 5. Relationships between water content and extracellular polymeric substances (log EPS), bacterial viability (log Live cells), β-D-xylosidase (log XYL) and β-D-glucosidase (log GLU). These linear regressions are obtained from linear mixed models results with water content as covariate for the surface (Sup) and hyporheic (Hyp) sediment during long-term drought in D and DS treatments (see Table 2).

DISCUSSION

This study exemplifies how a long-term drought (five months) compromises some ecosystem functions (such as those linked to organic matter degradation) and their recovery when rewetting. Also, the treatment with two flash storms showed how important sporadic precipitations that occur within a long-term drought can be for the recovery of ecosystem functions once the streambed is rewetted.

Long-term drought effects in surface and hyporheic sediments

The responses of microbial biomass and function to long-term drought were stronger and more significant in surface than in hyporheic sediments, likely because of the slightly higher water content maintained in the latter. As expected, five-month drought provoked a decline in algal biomass and bacterial viability in surface sediment, as well as a decrease in fungal biomass at the end of the drought period but did not affect bacterial density. This result partly coincides with other works, which observed a strong reduction of autotrophic and heterotrophic biomass when submitted to drying stress (Amalfitano & Fazi, 2008; Timoner et al., 2012), although an increase in prokaryote abundance was also reported in response to long-term drought but attributed to community composition changes (Pohlon et al., 2013).

Long-term drought caused a decrease in the active fraction of microbes, as is clearly shown in the case of bacteria. A decrease in fungal viability is also suggested, as it was measured as ergosterol content, considered a measure of living fungal biomass (Montgomery et al., 2000, but also consider Mille-Lindblom et al. (2004), indicating that a fraction of ergosterol can persist). The decrease in chlorophyll-a may also indicate a reduced photosynthetically active algal biomass as previously described for stream biofilm (Timoner et al., 2012). Among the three groups, the more resistant was fungi, as indicated by previous studies where fungal biomass was less affected by drought than bacteria probably because of their morphological life form with hyphal development that may cross air-filled soil pores to access nutrients and water (Barnard et al., 2013), making them less susceptible to predation and competition for substrates (Denef et al., 2001; Frank et al., 2015).

Besides the expected microbial strategies to survive desiccation, such as accumulation of compatible solutes for osmotic balance (Potts, 1994) or production of dormant life forms such as spores (Yuste et al., 2011), we reported a significant increase in EPS content from surface sediments, probably produced to cope with the low water availability (Roberson & Firestone, 1992). EPS are jelly substances (mainly composed of polysaccharides and peptides) that may enhance sediment physical stability, connecting soil aggregates for accessing distant resources, and also helping in resisting potential abrupt and strong rewetting (Flemming & Wingender,

2010). Fungi and bacteria colonising larger and smaller pores within sediment particles are able to form aggregates (as slime layers rich in EPS), which enhance water holding capacity and reduce moisture stress for the embedded microorganisms, drying more slowly (Denef et al., 2001).

These observed changes in the surface sediment microbial structure had consequences on its heterotrophic functioning, which was significantly reduced in the capacity to decompose simple polysaccharides usually originating from algal release, decay cells or cellulose material (GLU activity), or from hemi-cellulosic plant material (XYL activity). This decrease in extracellular enzyme activities could be due to: 1) the decrease in living bacteria (supported by the positive correlation of GLU and XYL with bacterial viability); 2) microorganisms investing a greater energy on resistant strategies (such as EPS production) rather than on organic matter decomposition and growth; 3) a decrease in organic matter availability, such as fewer algal exudates; 4) a reduction of enzyme and substrate diffusion when reducing soil moisture. For the latter, it has been shown that the reduction of soil moisture can limit enzyme and substrate diffusion (Liu et al., 2009) and enzyme efficiencies could be affected, possibly because processes such as enzyme immobilisation are increased while diffusion rates are reduced (Henry, 2013). On the other hand, the differential effect of drought on the distinct enzymes determined a greater capacity to use recalcitrant compounds in drought conditions (reduction of the recalcitrant index). Fabian et al. (2017) showed that the use of recalcitrant carbon compounds would be maximised with time since dry, due to the reduced labile organic matter sources in dried sediments. Moreover, in this study the maintenance of lignin degrading capability might be linked to the greater resistance of fungi than bacteria to drought, as fungi are the main responsible for this activity (Romaní et al., 2006).

The effect of long-term drought on microbial functioning was also shown by the reduction of leaf litter decay rate. In accord with our expectations, this was accompanied by the response of the extracellular enzymes which, although being not sharp, evidenced the reduced activity in the long-term drought, especially for hemicellulose degradation (XYL activity). Experiments on the effect of drought on leaf litter decomposition have shown a clear reduction in the decay rate (Abril et al., 2016) and significant effects were already detected even after 7 days of emersion (Mora-Gómez et al., 2018). Most studies in intermittent rivers showing the reduced breakdown rates during the drying streambed refer to the emersion effect (Foulquier et al., 2015) while in the present study leaf packs were embedded in the subsurface sediment. This position avoids the direct exposure to the air and probably maintains slightly higher water content and might be responsible for the less evident response of microbial function in leaf litter in comparison to the surface sediment to the long-term drought.

In contrast to the surface sediment and leaf litter, the microbial biomass and function at the hyporheic sediment was almost not affected by the long-term drought and showed higher microbial biomass, viability and extracellular enzyme activities than those of surface sediment, suggesting greater resistance. These results indicate that in the drying hyporheic some favourable conditions for microorganisms co-occur, such as some remaining water availability (2.54% water content while 0.54% on the surface, on average during the drying period) also combined with high oxygen availability (8.5 mg/L in contrast to the 0.07 mg/L in the wet hyporheic). Contrarily, at the water saturated hyporheic (Control treatment), the hypoxia conditions may determine the reduction of extracellular enzyme activities (GLU and XYL) probably also due to the reduction of living bacteria (Taylor et al., 2009).

The relevance of the slightly higher water content in the hyporheic in contrast to the drying surface in coping with desiccation is further suggested by the non-effect of the long-term drought on EPS content, indicating no need to invest energy on this resistance strategy. The moisture degree could help in the movement of solutes and cells which is strictly water dependent and essential for all the intra and extra-cellular reactions that support microbial life (Schimel et al., 2007; Frossard et al., 2012; Moyano et al., 2013) and may also allow fungi to move vertically through the sediment column, favouring the increase in fungal biomass in the hyporheic (Cornut et al., 2010). Overall, results emphasise that sediment moisture is essential for microorganisms, and may control microbial activity (Marxsen et al., 2010) as similarly found in arid soil ecosystems, where microbial growth is limited to specific wet periods (Kieft et al., 1987). In this regard, our results showed some variables being extremely sensitive to the decrease of water content (within a narrow range), which determined a reduction of bacterial viability and polysaccharidic enzyme activities, but an increase in EPS content (Fig. 5). However, these microbial responses to water content were particularly significant for the surface sediment and not as clear for the hyporheic. This suggests that, apart from sediment moisture, other characteristics of the physical environment such as the greater organic matter content and sediment texture and/or grains-aggregation of sediment particles may play a role on microbial resistance to drought observed at the hyporheos. Thus, microbial responses might vary when considering different types of sediments, or streams surrounded by different landscapes (Berggren et al., 2007; Berggren & del Giorgio 2015). In the present study differences in sediment texture were measured and higher content of clay (particles <20 µm) was found in the hyporheic (Table S2), which might retain water and a certain reserve of organic material (by formation of clay-organic complexes). At the hyporheic a positive interaction between sediment texture and moisture for microbial respiration and activity is

suggested as well as the physical protection of organic matter, promoting great conditions for heterotrophic viability (Moyano et al., 2013).

The relevance of storm episodes

The occurrence of occasional rains within the long-term drought period appears influential on the responses and maintenance of microbial functioning in streambed sediments. Despite low extracellular enzyme activities of the dried surface sediment and leaf litter, both were driven to a fast recovery when receiving the two storm episodes and, at the same time, bacterial viability and fungal biomass increased and EPS content decreased. In particular, the increase of XYL activity and leaf litter mass loss indicated a fast recovery of microbial functions linked to organic matter degradation. In contrast, the responses of storms at the hyporheic, already slightly moist and less affected by long-term drought, were much less evident.

The observed response might be linked to the increased water content (increasing from 0.36 to 2.73% in surface and from 2.86 to 5.48% in the hyporheic) which may further generate a release of carbon substrates accumulated during drought, inciting a microbial-driven mineralisation pulse (Barnard et al., 2014). The source of carbon that fuels the fast activity reactivation is controversial but it is more often related to: 1) a potential contribution of previously occluded (due to physical drought disturbance) carbon compounds (Blazewicz et al., 2014); 2) a release of microbial intracellular solutes (Fierer et al., 2003); 3) an increase in available dead microbial biomass (Kieft et al., 1987), becoming an available carbon source.

Our data suggests that the heterotrophic functional response rose with precipitation, probably due to microbial dead biomass available and to the dynamic slaking process of breaking down aggregates (rich in previously inaccessible organic substrates) created during the drought period (Dexter, 1988). In accordance with the slaking processes and considering both the porosity exclusion principle (Dexter, 1988) and the model of aggregate hierarchy (Tisdall & Oades, 1982), the larger aggregates created under dry conditions contain larger pores and are more sensitive to faster water infiltration, breaking down rapidly (upon fast rehydration) and liberating organic substrates which determine greater functional response (Denef et al., 2001), like the ones observed after the first flash storm (Fig. S4). In contrast, smaller aggregates (which previously composed the larger ones) are more resistant against the disruptive forces of slaking (Tisdall & Oades 1982, Denef et al., 2001), which is why the second fast rehydration might not cause the same microbial functional response size (second flash storm, Fig. S4).

Resilience upon rewetting

Studies on drought and rewetting evidenced that long-term stress might determine both the high metabolic cost of resistance, which is further translated as delayed structural

recovery, unexpected functional resilience (Fierer et al., 2003; Weaver et al., 2015), and rapid prokaryote colonization of overlaying water of the freshly re-wetted sediments (Fazi et al., 2008). Here we refer to resilience as the recovery after the disturbance (Shade et al., 2012) and was measured by analysing whether the rewetting phase drove the microbial variables to values similar to those of the water saturated condition (C). In the case of similarity between long-term drought (once rewetted) and control, we assumed that the variable considered had recovered from drought and thus was resilient. In the present study, surface dry sediment was the most affected and did not recover completely. One week of rewetting was not enough to recover algal biomass and two weeks of rewetting were not enough to recover the capacity to degrade simple polysaccharides (GLU activity). This suggests that, although fungal and bacterial viability were completely recovered, primary production (which may be a source of simple polysaccharides for heterotrophs) and heterotrophic degradation capacities were compromised. In contrast, the flash storms seemed crucial for providing resilience to the sediment and leaf litter since in the Dry-Storm treatment, microbial biomass, enzyme activities and litter decomposition rate were more rapidly and fully recovered. Interestingly, bacterial viability and EEAs (PHE and XYL) in the Dry-Storm hyporheic treatment not only achieved the same values as the control but also surpassed them, suggesting an enhancement of active microbial heterotrophs may be linked to flash peaks of organic matter availability driven by the rewetting of an extremely dry sediment (Fig.4). Also, the drastic reduction of EPS in the surface Dry-Storm indicated, once again, that microbial EPS production was highly sensitive to water availability (Figs. 4 and 5). Furthermore, since microbial persistence capacities might be compromised upon long-term exposure to drought, the microbes' responses reported in the present manuscript which took into consideration different micro-habitats might have implications for the microbial functioning under desiccation stress at the landscape scale.

CONCLUSIONS

Our results imply a link between physical sediment features and microbial activity under long-term drought, underlining the sensitivity of microbes to minimal changes in water content and the relevance of sediment moisture in reinforcing heterotrophic resistance to tolerate extended periods of water stress. Only two storm events were sufficient to make the effects of long-term drought barely noticeable for microbial functioning and the storms even stimulated carbon cycling processes and bacterial viability. Although a sediment column experiment does not describe the whole set of natural factors present in the environment, these results should be considered when predicting how long-term drought and flash storms could act on the carbon metabolism of temporary rivers.

CHAPTER 2

"Distinct responses from bacterial, archaeal and fungal streambed communities to severe hydrological disturbances"

This Chapter is submitted as:

Gionchetta G., Romaní A.M., Oliva F., Artigas J. Distinct responses from bacterial, archaeal and fungal streambed communities to severe hydrological disturbances. *Scientific Reports. In press.*

OVERVIEW

Stream microbes have been shown to possess heightened sensitivity to the intensified water stress, attributed to climate change, occurring in the Mediterranean Basin. Here, we investigate the effects of long-term drought (150 days), storms and rewetting (7 days) on the diversity and composition of archaea, bacteria and fungi inhabiting intermittent streambed sediment (surface and hyporheic) and buried leaves. Hydrological alterations modified the archaeal community's composition more than the bacterial ones, whereas fungi were the least affected. Throughout the experiment, archaeal communities colonizing sediments showed greater phylogenetic distance compared to those of bacteria and fungi, suggesting considerable adaptation to severe hydrological disturbances. The increase of classes' abundance, as Thermoplasmata within archaea, and Actinobacteria and Bacilli within bacteria, revealed signs of transitioning to a drought-favoured and soil-like community composition. Strikingly, we found that water return (as sporadic storms and rewetting) led to larger shifts in the surface microbial community composition and diversity than those observed during the drying phase. Besides, the microhabitat characteristics, such as the greater capacity of the hyporheic zone in keeping/conserving moisture, tended to modulate the ability of certain microbes (e.g. bacteria) to cope with severe hydrological disturbances.

BACKGROUND

Water scarcity is usual in Mediterranean freshwater ecosystems that endure the typical climate combination of high summer temperatures and extreme seasonal variation in rainfall patterns (Milano et al., 2013). The hydrology of intermittent Mediterranean streams consists of irregular flow, such as low or zero-flow between spring and summer, and low or regular flow during autumn and winter (Datry et al., 2017). Depending on the degree of intermittency, the flow regime can decrease or disappear and isolated pools can form along a stream's path. In this context, current global models predict temperature increases in the Mediterranean Basin, coupled with strengthened periods of drought and intense sparse flash storm episodes (IPCC, 2014; Ozturk et al., 2015). Contingent events, such as drought and rewetting, can alter resource availability and/or the physical environment, affecting the structure of a community, population or ecosystem. Based on their temporal patterns such unforeseen events have been classified as pulse, press and ramp disturbances (Lake, 2003, 2000; Ledger et al., 2012) and may promote distinct consequences on the microbial community. Pulses are generally short-term and steeply drawn disturbances (e.g. flood, rewetting or rainfall after long dry period), press disturbances describe conditions which get worse as the stressor persists, whereas ramps reflect disturbance whose strength increases in

time without an endpoint (e.g. droughts) (Lake, 2000). Despite flow intermittency being a natural part of the hydrologic cycle of intermittent streams, the adaptation of sediment microbiota to alternate dry-wet series could be modified depending on the duration, intensity and frequency of the disturbance (Lake, 2000; Zeglin, 2015).

Archaea, bacteria and fungi are the most important microbial groups with pivotal functions in the ecosystem. Their contribution to nutrient recycling and biogeochemical in-stream processes, necessitate exploring their respond to the long-term drought disturbance foreseen for intermittent Mediterranean streams. On one hand, studies reported that microbial communities inhabiting temporary streambeds (Marxsen et al., 2010; Zeglin, 2015) and soils (Meisner et al., 2015; Schmidt et al., 2018) change their functions and structure when submitted to prolonged dry conditions. On the other hand, recent research highlighted the observed capacity of microbes to develop resistance and drought legacy in the case of repeated perturbations (Allison and Goulden, 2017; Meisner et al., 2018; Wallenstein and Hall, 2012). These contrasting observed responses could be due to intrinsic differences in the microbial life strategies of bacteria, archaea and fungi, and/or in the occupied streambed microhabitat (such as surface and hyporheic zone sediments, or decomposing plant material accumulation). Also, responses may be modulated by the timing and severity of drought/rewetting episodes. In the present study, this knowledge gap is addressed by simulating different and severe climate change scenarios (i.e. long-term drought, punctual storms interrupting the drying process and abrupt rewetting) including the study of archaea, bacteria and fungi communities and three main streambed habitats (surface and hyporheic sediments and buried leaves), to provide a further step on predicting global change effects on streambed microbial ecology.

Focusing on the three microbial groups, the few recent studies available on archaea reported their ubiquitous presence in different environments (such as inland and marine sediments, hot springs, hydrothermal vents, solfataras, salt and soda lakes) and their great contribution in ammonia oxidation in terrestrial and/or freshwater sediment habitats (Buriankova, Iva, 2013; Compte-Port et al., 2017; Thion and Prosser, 2014). However, little is known about the diversity of archaea in inland freshwater ecosystems subjected to water stress conditions. At the same time, fungi (especially in dry sediment), are generally recognized as the most resistant group to drought, owing to their specific molecular and physiological traits (i.e., thicker and hydrophobic cell walls, mycelial growth, and spore motility, among others), which facilitate their adaptation to arid systems (Baschien et al., 2013; De Boer et al., 2005). Regarding bacteria, drought effects could lead to differing responses that mainly depend on habitat characteristics (such as sediment moisture, organic matter content and oxygen level),

hydrology history and drought duration and finally on the specific phylum being considered (Febria et al., 2012; Frossard et al., 2015; Zeglin, 2015). In spite of being the most studied microbes in temporary streams, knowledge on bacterial responses under prolonged streambed desiccation is still limited (Amalfitano and Fazi, 2008; Pohlon et al., 2013b; Timoner et al., 2014). According to the National Oceanic and Atmospheric Administration (Hoerling et al., 2012), a prolonged drought is defined as a dry period lasting more than three months. Such period, may transform temporary aquatic ecosystems into terrestrial, reducing the flow zones (usually in upstream branches) and increasing sediment heterogeneity and patchiness, which results in the formation of distinct microhabitats within the streambed. Therefore, the specific habitat where microbial communities develop is a key point that may determine their resistance and response to extreme hydrological intermittency. Greater pore-scale heterogeneity and aerobic or anaerobic microhabitats would promote the spread of highly diverse known and rare taxa (Datry et al., 2016). Free niches such as anoxic pools, oxygenated dry sediment, humid hyporheic layers and buried leaf packs, may induce the selection of certain microbial groups and opportunistic taxa which display physiological acclimation strategies (Cornut et al., 2010; Romaní et al., 2017; Wagner et al., 2014). Furthermore, the occurrence of sporadic storm events in the dry streambed may further modify the physical and chemical conditions of the streambed habitat (water, organic matter, availability of nutrients) with consequences on the microbial functioning (Gionchetta et al., 2019) and on community composition. Thus, the variety of micro-habitats and storm episodes would further differentiate the microbial trajectories of response, already recognized as multifaceted, between dry and wet phases.

Accordingly, the main objective of this study was to report on how microbial communities present in different sediment microhabitats (surface, hyporheic, and buried leaves) cope with the extreme alteration of hydrological intermittency in the freshwater ecosystem. Specifically, we assessed how archaeal, bacterial and fungal communities' structure and composition were affected by i) hydrological treatment effects, ii) treatment effects specific to microhabitat and iii) treatment effects in terms of taxonomic classes abundance variations over time. Microbial communities were sampled from sediment microcosms subjected to three treatments under controlled light and temperature conditions. After five months of drought simulation, all microcosms were subject to a one-week rewetting phase, simulating stream flow recovery and water table resumption. We expected that microbes inhabiting the surface sediment (as opposed to the other streambed habitats, i.e., the hyporheic zone and buried leaves), would respond more strongly to long-term drought due to the more direct exposure to desiccation effects. The moisture of the hyporheic zone and

that of buried leaves are expected to buffer the desiccation effects on microbes occupying these habitats. Besides this, among the microbial communities, we supposed that, given the specific molecular traits of fungi and the archaeal capacity of inhabiting extreme environments (Baschien et al., 2013; Brandt et al., 2015; Spang et al., 2015), fungi and archaea would be the most resistant groups to drought.

MATERIALS AND METHODS

Microcosms set-up

The experimental set-up was the same as that reported in Chapter 1 (Fig. 1). Briefly, the long-term drought experiment was performed in 12 streambed sediment microcosms randomly assigned to three treatments (n=4 per treatment): Control (C, maintained in wet, isolated pool-like conditions), Dry (D, 5-month drought), and Dry-Storms (DS, 5-month drought, including 2 flash storms). After five-month of drought treatment, all the microcosms were subjected to one-week rewetting, simulating stream flow recovery and water table resumption (Fig. 1).

The microcosms consisted of dark plastic cylinders (PVC), the base of which had been perforated with small holes (3 mm diameter) to allow water movement along the sediment column. Each microcosm had two small windows (ca. 1 cm^2) at a depth of 22 cm from the surface and on opposite sides of the microcosm to allow hyporheic sediment sampling. Oxygen micro-sensors (PreSens® sensor spots) were positioned on the surface and at 22 cm deep inside the microcosm (one per treatment) to monitor dissolved oxygen variations (Table S1A). The 12 microcosms were prepared *in situ* in early February 2016 and filled with streambed sediment from the "Santa Llúcia de Puigmal" stream, a headwater tributary of the Fluvià river (42.217637N, 2.401402E) displaying the typical Mediterranean discharge pattern (summer discharge reduction and flow recovery in autumn-winter). The water used for the entire experiment was collected from the same stream site. About 50-75 L of water was collected every two weeks, filtered through 0.2 µm pore size nylon filters (Whatman, Kent, UK) to ensure no microbes were added to microcosms when wet events were simulated or during the maintenance of the Control treatment, and kept in fresh conditions (4-10ºC). Before use, the filtered water was acclimated to room temperature for about two hours. Sediment stream sampling was performed in base flow conditions and the physicochemical parameters of the stream water were monitored to control that these parameters were not modified during the experiment (Table S2).

Once having created the sediment microcosms, and filled them with homogenized sediment, they were transported directly to the laboratory. In order to characterize the sediment

collected we measured the organic matter content and the grain size distribution. The organic matter content was measured as AFDW (Ash Free Dry Weight) for each 0.5 mL of sediment sample which was dried at 70°C during 72 h and burnt for 4 h at 450 °C using a muffle furnace (AAF 1100, Carbolite, UK). Overall, the organic matter content fluctuated between 0.009±0.001 and 0.015±0.002 grams of AFDW per gram of sediment dry weight (gAFDW/gDW) over the experimental time. In terms of grain size characterization, sediments were dominated by coarse fractions: sand and coarse-sand (Gionchetta et al., 2019). In each microcosm, five leaf litter bags (3x3 cm, mesh size 1 mm) containing 12 leaf circles (12 mm diameter) of *Populus nigra* L. (collected just after abscission and air-dried) were placed in the subsurface sediment layers (at 7-10 cm depth) and anchored with a thin string to enable bag extraction from the surface during sampling, thus avoiding any major disruption of the surface sediment. Then each microcosm was placed inside a larger plastic carboy (25 cm diameter, 30 cm depth), filled with fresh filtered river water (3L to each column) to maintain water saturation in all sediment microcosms during an acclimation period of 10 days before applying the three treatments (C, D, and DS). Water from all microcosms was renewed every two days with fresh filtered stream water to avoid nutrient depletion (Ylla et al., 2010).

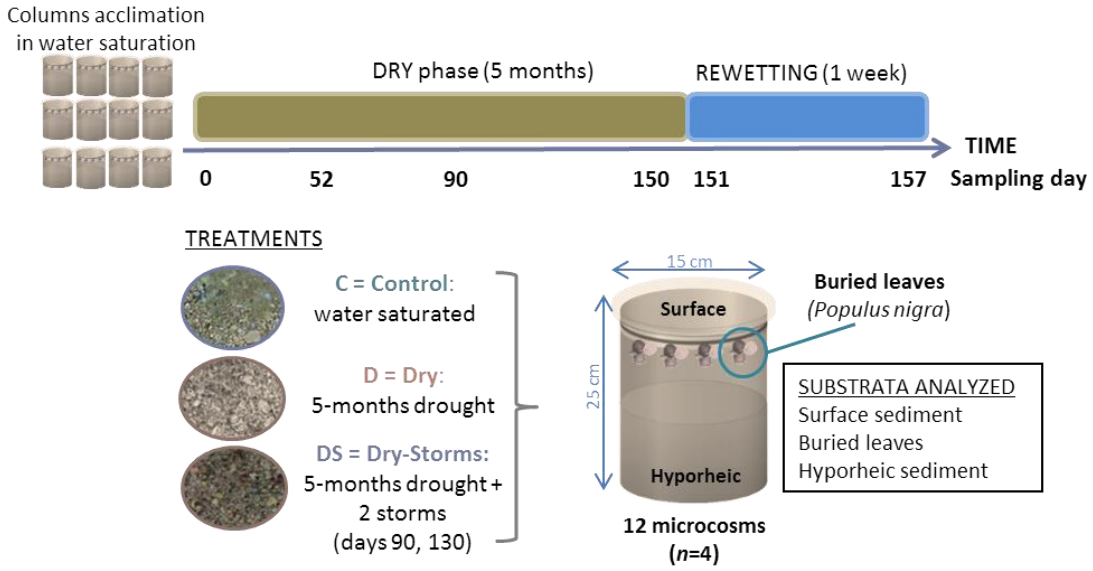


Figure 1 The experimental design.

Water replacement was performed by emptying the carboys and filling them with new water. All microcosms were placed in incubators (SCLAB, PGA 500) under controlled temperature and light conditions. Light conditions for the entire experiment were set at 12h/12h night/day, exposing the microcosms to a light irradiance (photosynthetic active radiation) of 150 $\mu Em^{-2} s^{-1}$ during the day and darkness during the night. The range of temperatures applied during the

experiment was set according to average values during the spring-summer period recorded in the watershed where sediment communities were collected (data 2005-2015 Idescat https://www.idescat.cat/pub/?id=aec&n=216&lang=en). Specifically, the day temperature range simulated the passage from late spring (25-29ºC over 6 weeks) to summer (30-31ºC for 12 weeks), while the night temperature was set 4 degrees below the maximum day value (Gionchetta et al., 2019).

After the first 10 days of microbial acclimation in the microcosms, the three treatments were applied. For the control treatment (C), the microcosms were maintained in saturated water conditions with a 4 cm layer of water at the top of the sediment, and the water was renewed twice a week. For the dry treatment (D), the microcosms were left to dry, the carboys were emptied, and no extra water was added for the next 150 days. For the Dry-Storms (DS) treatment, the microcosms were also left to dry as in Dry (D) treatment, but on days 90 and 130, 750 mL of diluted stream water (1:1, 0.2 µm filtered stream water:distilled water) was added over 30 min (simulating summer storms in that area, dataset 2005-2015 ACA – "Agència Catalana de l'Aigua"). During the experiment, the dissolved oxygen content was monitored at each sediment depth and for each treatment, while the water content was calculated as a percentage of water loss (%) obtained by the difference between the fresh and dry weights (Table S1B).

From day 150, all the microcosms were rewetted and maintained under saturated conditions with filtered stream water renewed every two days. Rewetting was performed by filling the outside carboys to simulate water table resumption with the filtration of water from the bottom of the microcosms (perforated base) and, at the same time, part of the water (1.5L) was poured into the top of the microcosms to simulate precipitation and allow the wetting of the entire sediment.

Sediment and buried leaves sampling

Surface sediment was sampled using a small syringe (2 cm diameter, 1x100 NORM-JECT®, Henke Sass Wolf, Germany) and attention was paid not to re-sample twice in the same place. Hyporheic sediment was collected with a thin spatula by opening one of the two windows created before filling the sediment containers, and then the window was closed and hermetically sealed to prevent deep sediment oxygenation (oxygen monitoring showed that hyporheic sampling did not cause any extra oxygenation, Table S1A).

The surface (0-5 cm) and hyporheic (20-25 cm) sediment was sampled from each microcosm replicate on days 0, 52, 90 and 150 during the drought phase, and after 24h (day 151) and 7 days (day 157) during the rewetting phase (Fig. 1). On each sampling date, treatment replicates (n=4) were pooled and split into two subsamples: 1mL for the analysis of microbial

community diversity (bacterial, archaeal, and fungal groups) and 4 mL for sediment characterization (Gionchetta et al., 2019). Concerning the buried leaves, one bag from each sediment microcosm was extracted on days 0 and 150 during the drought phase, and after 24h (day 151) and 7 days (day 157) of rewetting. The reduced frequency of sampling followed for the buried leaves was decided since a delayed and less strong response was expected for microbes inhabiting this habitat in comparison to those in surface and hyporheic sediment and also to minimize disturbance of the sediment columns (made when retrieving leaf bags). The sediment samples and leaves were immediately frozen at -20°C for further microbial diversity analyses.

High-throughput sequencing and sequence processing

Genomic DNA was extracted using the FastDNA™Spin Kit for Soils (MP Biomedicals, Irvine, CA), following the manufacturer's instructions, on sediment (0.5 g) and buried leaves (4 leaf circles of 1 cm in diameter) samples. Each sample was collected at every sampling time and combined for the distinct treatment (Fig. 1). The quantity and quality of the extracted DNA were measured spectrophotometrically (Nanodrop2000, Thermo Scientific™, Waltham, USA) and further assessed through 1% agarose gel electrophoresis. DNA extracts were stored at -20°C until sequencing analysis at the Roy J. Carver Biotechnology Center (University of Illinois, IL, US). The diversity of archaeal, bacterial and fungal communities was analysed using the primer pairs Arch 349F/Arch 806R (Takai and Horikoshi, 2000), V4_515F/V4_806R (Caporaso et al., 2011), and Euk_1391F/Euk Br(Medlin et al., 1988; Weisburg et al., 1991) using Illumina MiSeq technology. We run the IM-TORNADO pipeline version 2.0.3.2 (Jeraldo et al., 2014) to generate the archaeal, bacterial and fungal OTU tables and to assign taxonomy to the corresponding OTUs (≥ 97% sequence similarity). For bacterial and archaeal taxonomy assignment, the Ribosomal Database Project (RDP version 10) was used (Cole et al., 2014). For fungal taxonomy assignment, the Silva (version 128) (https://www.arb-silva.de/) database was used. Diversity analyses were performed using IM-TORNADO generated output BIOM table. The average values of sequencing depth were 66,535 for bacteria 19,654 for archaea and 19,889 for the total eukaryotes' DNA per sample.

Statistical Analyses

Rarefied OTU tables were generated by multiple rarefactions on bacterial, archaeal, and fungal BIOM tables using the *phyloseq* package by R software (R version 3.4.1). The rarefactions curves were generated (*rarefy_even_depth* function, *phyloseq* package) and then each rarefied OTU table was set to the smallest count of reads available for any sample in the table (9212 reads per sample for V4 and archaea, and 2831 reads per sample for fungi) in order to equalise sequencing depth samples. Alpha diversity (Shannon-Wiener and Richness

Chao1 indices) and the weighted Unifrac distance (measure of beta diversity) were calculated on the OTU tables (*phyloseq* package by R software, version 3.4.1). The weighted Unifrac distance matrix was estimated from the microbial phylogenetic trees (*distance* function in the *phyloseq* package, R version 3.4.1) and used as a proxy of the phylogenetic distance between the members of the communities analysed (Lozupone et al., 2011). Averaged weighted UniFrac distances were estimated for all time points to describe temporal variability in phylogenetic distance (Portillo et al., 2012) for the bacterial, archaeal and fungal communities inhabiting the surface and hyporheic sediments and buried leaves. The sequencing analysis of the archaeal communities inhabiting the buried leaves was completely unsuccessful in most of the samples and so it was excluded from the study.

In order to estimate the differences depending on the three experimental factors (Treatment, TR; Time, TI; Habitats, HAB) PERMANOVA analyses based on 9999 permutation have been run for the diversity indices and phylogenetic distance, addressing the problem of potential autocorrelation (PRIMER 6 version 6.1.11 & PERMANOVA+ version 1.0.1). Being significant the differences observed between Habitats (as surface and hyporheic sediments and buried leaves), further PERMANOVA analyses specific for each habitat have been assessed (Treatment, TR and Time, TI as factors; PRIMER 6 version 6.1.11 & PERMANOVA+ version 1.0.1). Furthermore, aware of the potential problem of combining samples the diversity results were further analysed by Repeated Measures to address the potential pseudo-replication (method: Scaled Identity; results shown in the Supplementary Information S1) (IBM SPSS STATISTICS 25 software (SPSS Inc., USA)). For the community composition analysis, Principal Coordinates Analysis (PCO) provided an ordination of the bacterial, archaeal and fungal communities in the different habitats and subjected to the different treatments in a factorial map based on the scores of the first two principal components. The abundance matrices used for ordination analyses were previously fourth root transformed and distance matrices were obtained based on Bray-Curtis dissimilarity. To the PCO analyses the permutation multivariate analysis of variance PERMANOVA analyses were combined in order to elucidate eventual community composition differences between treatments (C, D, and DS), types of habitats (sediment surface, hyporheic, buried leaves) and their interaction, considering 9999 permutations (PRIMER 6 version 6.1.11 & PERMANOVA+ version 1.0.1). The shifts in the archaeal, bacterial, and fungal classes abundance (with relative abundance >5%) between C, D, and DS treatments were tested by one-way ANOVA (Fixed factor = treatment (C, D, DS) and represented by box plots including all the time (from 0 to 157 days)) using IBM SPSS STATISTICS 25 software (SPSS Inc., USA). Finally, the percentage of shared OTUs (%) between treatments and for each microbial group (bacteria, archaea, fungi) were detected by the

average value obtained by Venn diagrams study (data not shown), using the Venn Diagram library (Venny 2.1, Oliveros (2007–2015) http://bioinfogp.cnb.csic.es/tools/venny/index.html).

Data availability

Sequencing data were deposited in the NCBI (National Center for Biotechnology Information) under of SRA accession number (Sequence Read Archive): PRJNA507856.

OUTCOMES

Initial microbial communities

The taxonomic composition of the initial bacterial, archaeal and fungal communities showed no significant differences between treatments (Table S3, Fig. S1). In terms of phylum and class composition, the initial bacterial communities were dominated by Proteobacteria (50% of relative abundance in sediments, and around 65% in leaves, Fig. S1A). Within the Proteobacteria group, Alphaproteobacteria was among the most abundant classes in both types of sediment (20% of relative abundance) while Gammaproteobacteria was among the most abundant in leaves (30% of relative abundance, data not shown). In the archaeal communities, Thaumarchaeota was the dominant phylum (75% relative abundance in the surface and hyporheic sediments, Fig. S1B). Within the Thaumarchaeota phylum, the Nitrosopumilales and Nitrososphaerales classes were the most abundant (44% and 34% of relative abundance respectively, data not shown). Finally, Ascomycota, Basidiomycota and Cryptomycota were the most dominant fungal phyla in the sediments with high phyla and class variability between treatments in the surface and hyporheic sediment (Fig. S1C). The fungal community inhabiting the leaves was dominated by Ascomycota (80% of relative abundance, Fig. S1C) and Dothideomycetes class dominance (78% of relative abundance, data not shown). On the other hand, initial diversity calculated as Shannon-Wiener and Richness indices was higher, as absolute values, for bacteria compared to that of archaea and fungi (Table S4).

Responses of microbial community structure to treatments across habitats

The structure of the microbial communities was apparently shaped by the treatments, but the magnitude of these changes mainly depended on the type of streambed habitat considered and the experimental time (surface and hyporheic sediments or buried leaves, Table 1, Table S4). In sediments, bacterial richness and diversity were affected by treatments interacting with time and by habitat factor, with decreasing values occurring at the surface in the D treatment at the end of the long-term drought and beginning of rewetting (Table 1, t150-t151 in Table S4). In leaves, bacterial diversity and richness were also reduced by treatments, especially in the D community at the end of the long dry phase (Table 1, Table S4). Archaeal richness and diversity were affected by treatments and habitat factors, where richness values decreased in

D and DS treatments of the surface sediment those of the hyporheic sediment were more consistent during the experimental drying time (Table 1, Table S4). Fungal were the least affected and only fungal diversity of the hyporheic C and D treatments was reduced when rewetted the sediments (Table 1 and Table S4). The percentage of shared OTUs between treatments reported averaged values of 7.21%, 6.87% and 0%; and 8.67%, 6.23% and 0% for surface and hyporheic sediment and bacteria, archaea and fungi, respectively (Fig. S2). In the buried leaves, the percentage of shared OTUs between treatments was in average 7.05% and 3.75% for bacteria and fungi, respectively (Fig. S2). The greatest time variation in shared OTUs was that observed for archaea in surface sediments which showed a gradual decrease during drought and an increase when rewetting (Fig. S2). Shared OTUs in bacteria inhabiting surface sediment followed a similar but less marked trend to that of archaea (Fig. S2). In the hyporheic sediment, the percentage of shared OTUs was more constant over time while in buried leaves it decreased by the end of the long-term drought and did not recover during the wet phase (Fig. S2).

Table 1. Significance of PERMANOVA results (p-values) for Shannon-Wiener (H), Richness (S) indices (raw values are presented in Table S4) and for the phylogenetic Unifrac distance. The diversity and distance matrices were calculated from the OTU table of bacterial, archaeal and fungal assemblages inhabiting three distinct habitats, indicated as: SUR, surface sediment, HYP, hyporheic sediment, LEAVES, buried leaves. A) Results from PERMANOVA models including three factors (Treatment, TR; Time, TI; Habitat, HAB).

A.	SHANNON (H)			RICHNESS (S)			UNIFRAC		
	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi
TR	*0.061*	**0.029**	**0.044**	0.245	**0.005**	0.425	**0.001**	0.443	0.261
TI	**0.002**	0.227	*0.091*	**<0.001**	0.486	0.128	0.265	0.231	0.907
HAB	**0.004**	**0.017**	**0.052**	**0.002**	**0.049**	0.223	**0.001**	**0.009**	0.302
TRxTI	**0.043**	0.146	0.759	**0.002**	0.672	0.289	0.540	0.451	0.778
TRxHAB	0.196	0.778	*0.070*	**0.004**	0.221	0.131	**0.002**	**0.056**	**0.037**
TIxHAB	*0.098*	0.065	0.262	**0.004**	0.110	0.273	0.952	0.180	0.778

B.	SHANNON (H)			RICHNESS (S)			UNIFRAC		
SUR	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi
TR	0.310	0.472	0.110	0.412	0.170	0.347	**0.001**	0.113	0.541
TI	*0.073*	0.421	0.188	**0.005**	0.196	0.365	0.781	0.193	0.918
HYP	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi
TR	0.250	**0.044**	**0.010**	0.145	**0.002**	0.366	0.717	0.176	**0.001**
TI	**0.050**	0.075	**0.042**	**0.013**	0.163	0.246	**0.038**	0.184	**0.034**
LEAVES	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi	Bacteria	Archaea	Fungi
TR	**0.004**	*na*	0.470	**0.007**	*na*	0.838	0.971	*na*	0.339
TI	0.170	*na*	0.348	**0.039**	*na*	*0.096*	**0.033**	*na*	0.347

Here, the *Habitat* factor includes surface and hyporheic sediment habitats (and not buried leaves) since the sampling time sequence was shorter in the case of leaves. B) Results from PERMANOVA considering each habitat individually and including two factors (Treatment, TR and Time, TI). Significant differences ($p<0.05$) are indicated in bold while those at the limit of significance ($p<0.1$) are indicated in italics, and the *na* stated for not available data.

Responses of microbial community composition to treatments across habitats

In terms of composition, the bacterial and archaeal communities showed significant differences between treatments and types of habitat and both factors interact (Fig. S3, Table S5). The fungal communities also responded significantly to the types of streambed microhabitats and to their interaction with the treatments (Fig. S3, Table S5). To better highlight the effects of treatments, further analyses for each habitat (surface and hyporheic sediments or buried leaves) were independently carried out (Fig. 2, Table 2).

Table 2. Resulted *p*-values of PERMANOVA analyses assessed for each microbial community (bacteria, archaea, fungi) inhabiting the three habitats: surface sediment, hyporheic sediment and buried leaves. Significant differences (p<0.05) between *treatments* (C, Control; D, Dry; DS, Dry-Storms), and relative pairwise comparisons, are indicated in bold while those at the limit of significance (p<0.1) are indicated in italics.

SURFACE SED.	Bacteria	Archaea	Fungi
Treatments	**0.004**	**0.014**	**0.005**
C, D	**0.002**	**0.021**	**0.026**
C, DS	**0.002**	**0.004**	**0.016**
D, DS	0.438	0.814	0.351

HYPORHEIC SED.	Bacteria	Archaea	Fungi
Treatment	**0.001**	**0.003**	0.197
C, D	**0.002**	**0.003**	-
C, DS	**0.016**	*0.063*	-
D, DS	*0.082*	**0.035**	-

BURIED LEAVES	Bacteria	Archaea	Fungi
Treatment	**0.018**	n.a.	0.667
C, D	**0.030**	n.a.	-
C, DS	*0.067*	n.a.	-
D, DS	0.506	n.a.	-

Bacterial communities inhabiting the surface and hyporheic sediments in the D and DS treatments were significantly different to those in C (Fig. 2A, 2B, Table 2). Furthermore, the bacterial communities colonizing the leaves buried in the dry sediments (D and DS treatments) were significantly different to those found in the C condition (Fig. 2C, Table 2; at the limit of significance in the case of DS, p=0.06). In the case of archaea, the surface sediment communities in the D and DS treatments were significantly different from C (Fig. 2D, Table 2).

Community composition trajectories during the experiment indicated that, in both D and DS, the bacterial communities in surface sediment was gradually modified during the drying process, and was eventually differed from the C community. However, the rewetting in D and the second storm in DS, determined the largest community changes when compared to the C community (Fig. 2A). Although a shorter time sequence was presented, similar trajectories were observed for bacteria in the buried leaves (Fig. 2C). At the hyporheic zone and upon rewetting, the bacterial communities inhabiting the DS returned closer to a community

composition like that in C (T157, Fig. 2B), whereas the D community remained separated from C and DS (Fig. 2B).

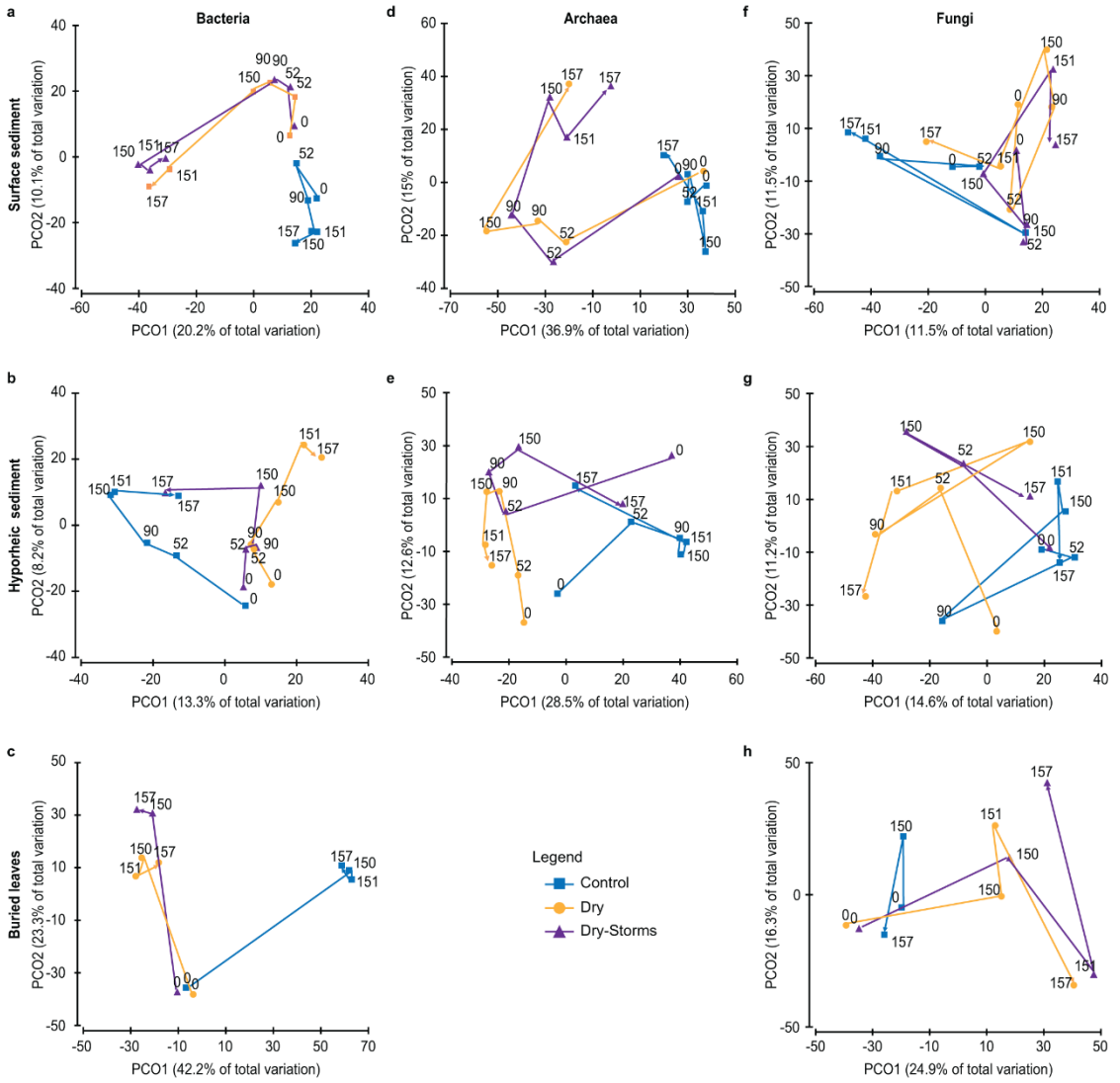


Figure 2. PCO from bacterial, archaeal and fungal OTUs, inhabiting the three habitats, indicated as: surface sediment; hyporheic sediment; buried leaves. The arrows indicate the temporal evolution. The colour pattern is indicated in the legend for the three treatments: Control (C); Dry (D); Dry-Storms (DS).

Overall, the bacteria inhabiting the surface sediment in the dry conditions (D and DS) showed greater phylogenetic distances compared to that of C, throughout the experiment (Fig. 3A, Table 1B). In the case of the surface bacteria in D, phylogenetic separation was particularly evident just after rewetting (day 151, Fig. 3A). Regarding the hyporheic zone and buried leaves, bacterial communities showed lower phylogenetic distance values overall, apparently unaltered between treatments (Fig. 3B and 3G, Table 1B).

As in the case of bacteria, rewetting and the second storm determined significant changes in the archaeal community compositions of D and DS, respectively (Fig. 2D, Table 2). But also, in contrast to the example of bacteria, in the surface sediment the archaeal community trajectories of the D and DS communities were split from that of C after 52 days of drying (Fig. 2D, Table 2). In the hyporheic sediment, significant differences were found between C and both the D and DS archaeal communities (Fig. 2E and Table 2; at the limit of significance in the case of DS, p=0.06). Furthermore, the hyporheic D community was significantly different to that of DS, which, at the end of the wet phase (DS day 157, Fig. 2E, Table 2), was clearly trending towards recovering its composition and being closer to C. In terms of phylogenetic distance, the archaea inhabiting the surface sediment showed higher distance values and greater effect of treatments compared to those in the hyporheic zone (Fig. 3C, 3D, Table 1A, Habitats and Treatment x Habitats effects) and, above all, to those of bacteria (Fig. 3A, 3B).

The fungal communities inhabiting the surface sediment showed significant effects of treatment on their composition, whereas no effect was found in the hyporheic sediment and buried leaves (Table 2). In particular, in the surface, treatment C was significantly different from either D or DS (Fig. 2F, Table 2). The phylogenetic distance of the fungal community inhabiting the surface sediment and buried leaves detected no significant effect of time or treatment (Fig. 3E and 3H Table 1B). However, in the hyporheic sediment, the phylogenetic distance was significantly affected by treatment and experimental time, highest values being measured in the DS treatment (Fig. 3F, Table 1B). Most of the communities, mainly bacteria and archaea, started out very similar at time zero but the dry and dry-storms treatments apparently changed the communities even after seven days of wetting to control levels (Fig. 2A, B, C, D, H). This consistent trajectories of change over time may correspond to the legacies and lack of prompt resilience in the dry treatment reported in the functional study (Gionchetta et al., 2019; Chapter 1).

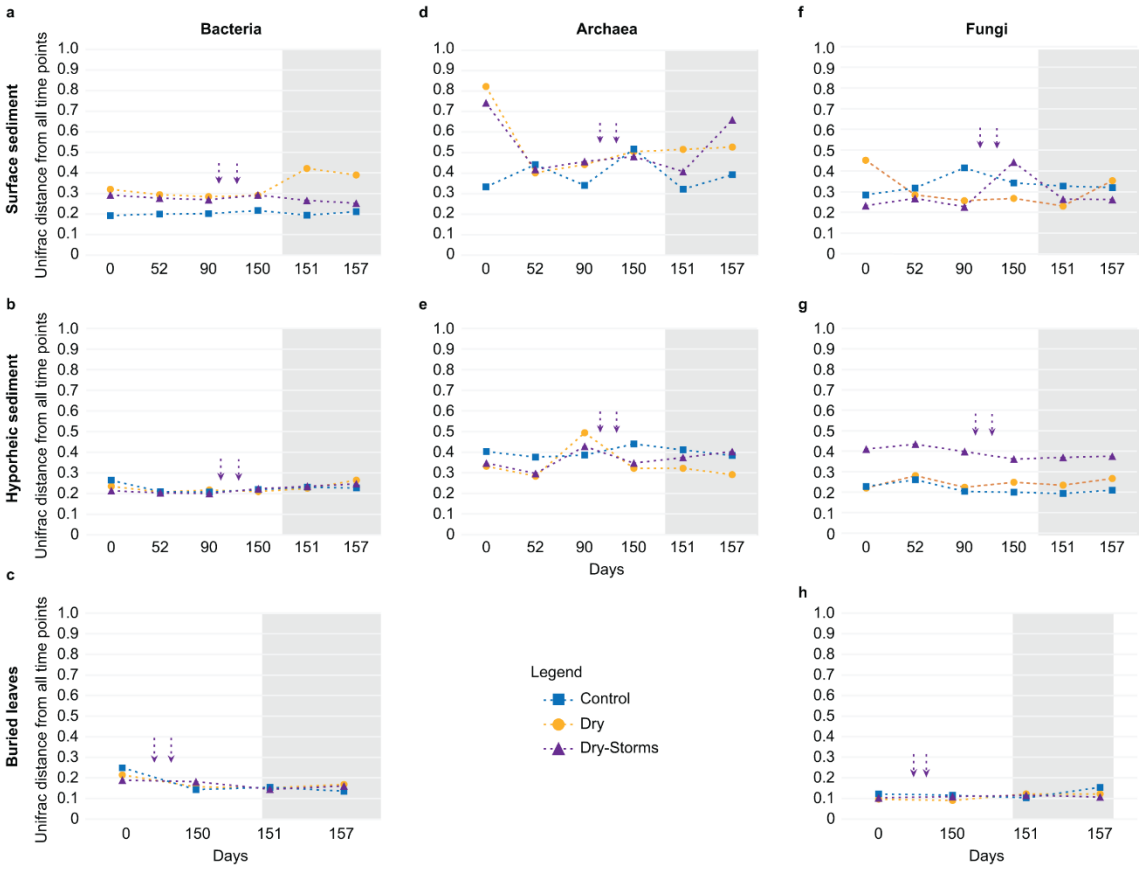


Figure 3. Time changes in the average weighted UniFrac distances between each sample and all other samples from the same treatment for bacterial, archaeal and fungal communities inhabiting surface and hyporheic sediments and buried leaves. Higher points on the y-axis indicate samples with greater phylogenetic distances with respect to the rest of the samples from the same treatment. Shaded area indicates the rewetting phase. The colour pattern is indicated in the legend for the three treatments: Control (C); Dry (D); Dry-Storms (DS). Vertical purple arrows indicate when storms occurred for the DS treatment.

Variation in microbial classes' abundances due to treatments

Each microbial group considered showed a specific sensitivity to drought and watering events (as storms and rewetting) in terms of taxonomic composition, though these changes strongly depended also on the habitat considered. Regarding bacteria, overall the D and DS treatments resulted in an increase in the relative abundance of Actinobacteria and Alpha-proteobacteria and a decrease in Delta-proteobacteria in the surface sediments compared to those of the C treatment (Fig. 4A). Similarly, in the hyporheic sediment, the D and DS treatments determined an increase in Actinobacteria and a decrease in Delta-proteobacteria, with significant changes for D treatment (Fig. 4B). In the case of the bacterial groups inhabiting the buried leaves, the Alpha-proteobacteria and Sphingobacteria classes increased significantly in D and DS, whereas Bacteroidia sharply decreased (Fig. 4C). Regarding archaea, changes at

class level in D and DS treatments comparing to C were observed in surface and hyporheic sediments (Fig. 4D, 4E). Thermoplasmata was strongly enhanced in D and DS communities during drought, while Nitrosopumilales and Methanobacteria decreased. Finally, fungal diversity at class level did not show any differences between treatments since high variability in community composition were already observed in the C condition (Fig. S4 and S6).

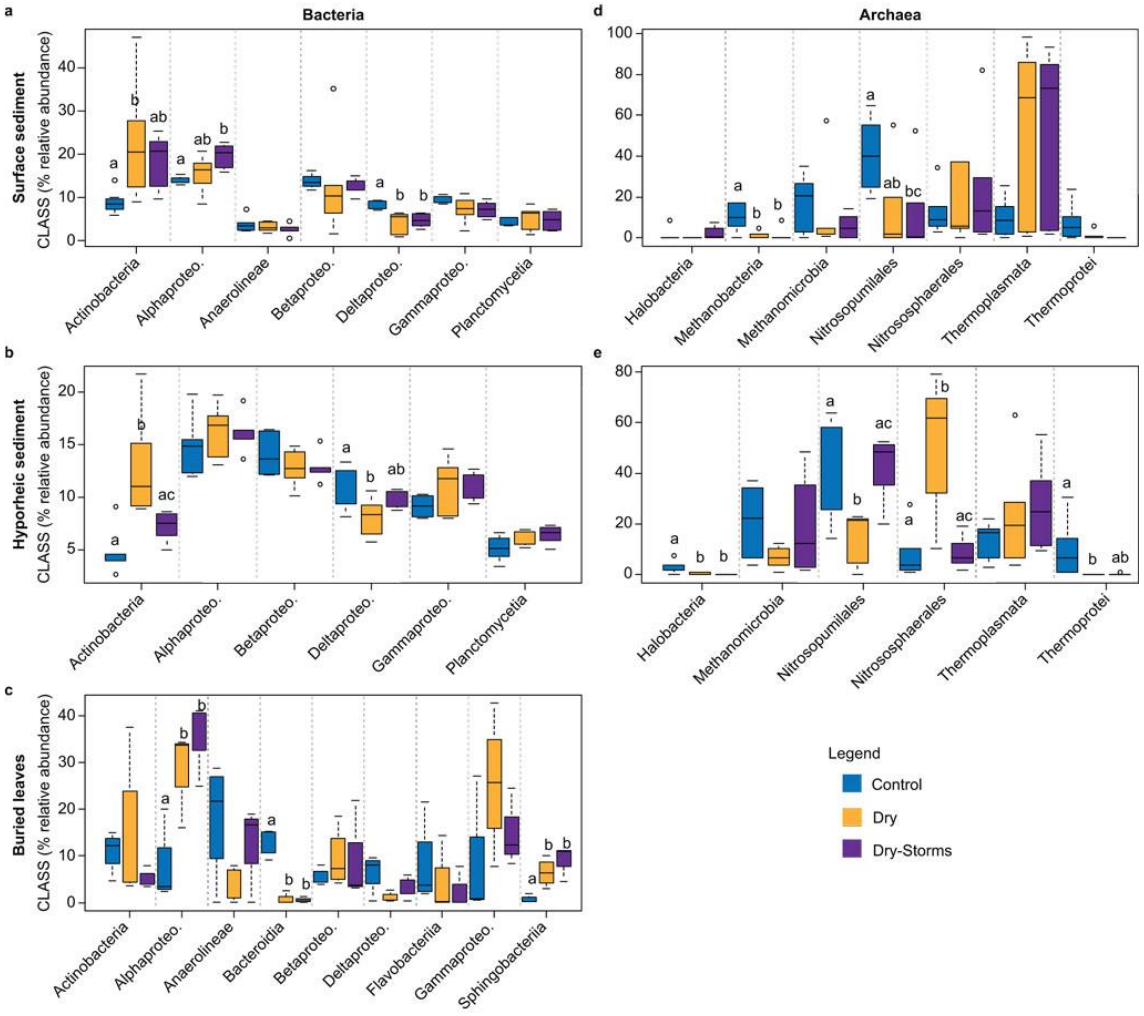


Figure 4. Boxplots for bacterial and archaeal class variability (percentage of relative abundance) considering only those classes reporting abundance higher than 5% throughout the entire experimental period. To better visualize where changes occurred, each axis is on a different scale of abundance. The three habitats are indicated as: surface sediment; hyporheic sediment; buried leaves. The colour pattern is indicated in the legend for the three treatments: Control (C); Dry (D); Dry-Storms (DS).

The observation of the taxonomic classes' relative abundances revealed fluctuations over the experimental time. Bacterial class abundance, in the surface sediment under the long-term drought condition, showed that Actinobacteria gradually increased by up to 20% compared to

the beginning of the experiment (at day 150 in D, Fig. S5A). Within Actinobacteria, the abundance of *Arthrobacter* and the *Nocardioides* genera (both gram positive Actinomycetales) during the drought phase (they corresponded respectively to 3% and 2% of the total genera presented at the end of the drought phase, D at day 150, data not shown). The first 24 hours of rewetting saw a peak in the increase of Actinobacteria and Bacilli in contrast to a Proteobacteria (Delta-, Beta- and Gamma-) reduction in the D bacterial communities inhabiting the surface sediment in D (on day 151, Fig. S5A). However, at the end of the wet phase, the proportion of class abundance returned to levels similar to the initial ones, albeit with a two-fold relative abundance of Beta-proteobacteria (D on day 157, Fig. S5A). In the DS treatment, temporal changes in the relative abundance of the classes were less evident, showing an increase of Actinobacteria (6%) at the end of the drought phase (DS on day 150, Fig. S5A). In the hyporheic sediment, the proportion of the main bacterial classes was like that of the surface, albeit with a dominance of Alpha-proteobacteria and Beta-proteobacteria, and time changes were not observed. However, overall Actinobacteria abundance tended to be greater in D compared to C and DS (Fig. S5B).

The abundance of Alpha-proteobacteria class, colonizing the buried leaves under D and DS treatments, increased about 34% at the end of the dry phase compared to the initial values (from time 0 to time 150, Fig. S5C). Rewetting did not cause any major changes in the composition of the bacterial communities inhabiting the hyporheic zone or the buried leaves, but the increase in abundance of Actinobacteria at the end of the wet phase (day 157 in D, Fig. S5B, Fig. S5C).

Concerning archaea communities, D and DS treatments experienced a reduction of Nitrosopumilales and Methanobacteria throughout the experiment in surface sediments (Figs. 4D and S5D) while Nitrososphaerales decreased with the drought phase but not the wet (Fig. S5D). Thermoplasmata was considerably enhanced in the surface sediment, becoming dominant in the D and DS communities during the drought phase (>90%, Fig. 4D and S5D). *Ferroplasma* and especially *Thermogymnomonas* were the Thermoplasmata genera that expressed the greatest increase in abundance during the long-term drought phase (data not shown). Nevertheless, *Nitrosopumilus* (Nitrososphaerales class) and *Nitrososphaera* (Nitrosopumilales class) were considerably reduced during the drought phase (data not shown). Shifts in the abundance of classes were also observed during rewetting. In particular, on day 157 in the D and DS surface groups, Methanomicrobia (mainly *Methanolinea* genus) and Nitrososphaerales increased their abundance by 60% and 40%, respectively, while Thermoplasmata largely decreased (<10%, D and DS at day 157, Fig. S5D).

In the hyporheic sediment in treatment D, Nitrososphaerales clearly increased during both the drought and wet phases (Figs. 4E and S5E), but Nitrosopumilales significantly decreased as the experiment progressed, almost disappearing by the end of the wet phase (Fig. 4E; day 157, Fig. S5E). The relative abundance of the archaeal classes overall was steadier in the DS hyporheic sediment (Figs. 4E and S5E).

The fungal communities inhabiting the buried leaves showed that Dothideomycetes and Tremellomycetes (mainly *Cystofilobasidiales* and *Tremellales* genera) increased in treatment D during the drought phase, while Leotiomycetes were favoured by the DS treatment (Figs. S4 and S6). However, even by the end of the rewetting phase, the high variability of the fungal community in the leaves was still evident. Agaricomycetes dominated the C treatment whereas Hymenoscyphus was dominant in D and Eurotiomycetes in DS (on day 157, Fig. S6).

DISCUSSION

The present simulation of hydrological disturbances including a five-month drought, flash storms and rewetting, showed distinct community responses of the streambed bacteria, archaea and fungi. Likewise, different responses from the microbial communities were observed for the diverse streambed microhabitats: surface sediment, hyporheic sediment and buried leaves. Despite the potential contribution given by the present study, we should point out that the pooling of replicates applied may affect our observations (i.e. reducing variability) and thus it requires caution in getting strong conclusions from the obtained results, although proper statistical tools have been applied.

When subjected to the long-term drought, bacteria and archaea showed a similar transition to more terrestrial communities in terms of class relative abundance, but the way of response used varied. Under different environmental and hydrological conditions, most probably the resources competition dynamics determined the differences observed in the community's responses. Bacteria community gradually modified from the outset without any drastic changes being observed, whereas archaea presented a larger shift in the community composition, already at day 52 and onward. Surprisingly, the archaeal community changed more than that of the bacteria, but both microbial groups showed increments of some drought-favoured taxa classes abundances, which were also the most present in terms of OTU sequences. Large variation in the abundance of certain classes was reported among archaea community. Specifically and similarly to previous studies, Thermoplasmata class (Euryarchaeota phylum) dominated the dry phase (Compte-Port et al., 2017; Monard et al., 2016). Within this class, the *Thermogymnomonas* genus dominated in the surface sediment of

the Dry treatment (88% relative abundance) and it was probably due to its recognized capacity to cope with osmotic and drought stress (Itoh et al., 2007).

On the other hand, Nitrosopumilales and Nitrososphaerales (Thaumarchaeota phylum) considerably reduced their presence under the long desiccation, and this trend of abundance response was in contrast to previous studies which identified Nistrososphaerales as dominant in many dry streams (Monard et al., 2016). Furthermore, within this class the *Nitrosphaera* genus, known as an important player in the nitrification of intermittent streams (Merbt et al., 2016; Stahl and de la Torre, 2012), was extremely reduced and this would potentially lead to consequences for the streambed nitrogen cycle (i.e., reducing nitrate leaching). The discrepancies in the classes abundance fluctuation observed between literature of field-study results and our experimental findings could be explained by an additional laboratory stress (e.g. simplified community interactions between biotic and abiotic factors due to the exclusion of environmental inputs) or could be due to the specificity of the microbial community inhabiting the stream sediment sampled and inoculated into the columns.

In the case of bacteria, the long-term drought caused some changes in the composition as shown by the increasing abundance of certain taxa, especially within the surface sediment. The spread of Actinobacteria and Firmicutes (mainly Bacilli), characteristic taxa of arid and soil environments (Aslam et al., 2016; Barnard et al., 2013; Schimel, J., 2007) observed during the drought, suggested the beginning of a terrestrial transition. Actinobacteria have already been described as being able to survive lengthy dry periods (Bouskill et al., 2013) thanks to the strong, thick and interlinked peptidoglycan cell wall of gram-positive bacteria (i.e., Actinobacteria and Firmicutes) and their capacity to form colonies which often grow extensive mycelia (like fungi) allowing the habitat to be explored, water sought and appearance of dormant cells (Schimel, J., 2007; Zhou et al., 2016). More specifically, in this study *Arthrobacter* genus (Actinobacteria class, Actinomycetales order) dominated the desiccation period and this might have been facilitated by its capacity to resist desiccation and starvation(Potts, 1994) and its ability to metabolize aromatic compounds as its sole carbon source (Kallimanis et al., 2007; Stevenson and Hallsworth, 2014). Desiccation process combined with external inputs (e.g. leaves fall during late summer-autumn) generally increase the accumulation of lignin-like compounds in the streambed (Ylla et al., 2012); in this experiment, the exclusion of external inputs, suggested that the organic matter got higher aromaticity due to its persistence in the sediment where it was accumulated during the desiccation period.

In contrast to the surface sediment, bacterial community inhabiting the hyporheic sediment resisted better the long-term drought than the superficial one, and the phylogenetic distances and the shared OTUs between treatments were apparently less influenced by the hydrological

changes throughout the experiment. The responses of fungal communities' composition to the experimental conditions resulted difficult to interpret since it was the most variable group from the beginning of the experiment and onward. The large variability observed in the first place (at the initial time) could have enhanced the diversification of taxa present and the associated capacities to cope with disturbances. Also, the high number of micro-niches potentially created during the drought phase might have determined the presence of dormant spores being germinated during the course of the experiment, and vice versa, thus increasing the variability observed in the fungal group at each experimental time. Similar to previous studies, the majority of Dothideomycetes, especially those observed in the buried leaves, highlighted their amphibian nature and their preference for colonizing leaf litter and plant material (Kohout et al., 2012; Suberkropp et al., 1983). Despite the large dynamics in the fungal communities, in the surface sediment they were significantly affected by the long-term drought, similar to what was observed for the bacteria and archaea.

The integrated vision of our findings by including all experimental phases (drought, storms and rewetting) apparently revealed that the largest shift in community structure and composition occurred under the sporadic storms or rewetting phase which, in turn, seemed to influence the microbial biodiversity, especially for the communities colonizing the surface sediment. Specifically, the consequences of the wet events refer to the changes in the relative abundance of classes, the reduction in OTUs' richness and the abrupt variation in community similarities, especially from Dry bacteria and Dry-Storms archaea communities.

Generally, a rewetting phase subsequent to a dry period provokes a cascade of physical and biological events (Manzoni et al., 2012), resulting in enhanced heterotrophic microorganisms respiration (Meisner et al., 2015), dormant cells awaking (Aanderud et al., 2016; Jones and Lennon, 2010), nutrient mineralization and also the mobilization of carbon protected in aggregates (as after storm events), and the release of intracellular osmolytes (Borken and Matzner, 2009; Schimel, J., 2007; Timoner et al., 2014). In this study, the arrival of water after a long dry period might have broken up sediment aggregates and compromised the stability achieved with the extracellular polymeric substances produced during the drought phase (Gionchetta et al., 2019), or caused the cell wall breakage (Kieft et al., 1987) of certain microbes which did not dispose of the appropriate osmolytes to compensate for the rapid osmotic variations in the media (Wood et al., 2001). These physicochemical consequences, combined with the gravitational water movement, might have determined the release of intact aggregates as well as the release of already dead cells stuck in the sediment and thus have determined a change in the composition of the community. In parallel, the release of nutrients within the rewetted sediment might have triggered the spread of certain microbial

groups metabolically adapted to the osmotically changed conditions, for instance the archaeal Methanomicrobia, Nitrosopumilales and Nitrososphaerales inhabiting the surface sediment. These groups may, in turn, promote processes related to both carbon and nitrogen cycles (Kerou et al., 2016; Stieglmeier et al., 2014). Something similar apparently occurred for the bacteria, where Proteobacteria (mainly Alpha- and Beta-proteobacteria) increased their abundance in the surface sediment after rewetting in the community from the drought treatment. Interestingly, they have already been identified as a group that responds positively to rewetting (Meisner et al., 2017, 2015; Pohlon et al., 2013b).

On the other hand, even though the storm events seemed to modify prokaryote diversity, mainly in the surface sediment, and barely influenced their composition, hidden consequences from the precipitation events were revealed when comparing the responses of the communities from the dried sediments (Dry treatment) with those previously wetted by storms (Dry-Storms treatment) at the end of rewetting. Concretely, the bacteria and archaea inhabiting the hyporheic sediments in the Dry-Storms condition returned to the relative abundance of the classes and similar community composition to that seen at the beginning. In parallel, a comparable trend was reported in Dry-Storms surface bacteria although it was evident only in terms of class abundance. These observations suggested that the simulated sporadic storms interrupting the long dry period could have promoted and boosted microbial recovery, at least in terms of classes' composition.

CONCLUSIONS

As hypothesized, the microhabitat characteristics emerged as a relevant factor that might influence the microbial community composition under both water stress and rewetting situations. The surface sediment was the habitat most affected by the hydrological disturbances, followed by the buried leaves and hyporheic sediment, where the latter may buffer the hydrologic impacts up to a certain point. Bacterial communities, more than the archaeal ones, inhabiting the hyporheic sediment, remained almost unchanged in terms of diversity and class abundances, probably fostered by the combination of higher humidity, a higher fraction of fine particles (Gionchetta et al., 2019) and, therefore, resource retention (Moyano et al., 2012).

The higher moisture of the hyporheic zone compared to that of surface sediment (3.41% and 0.97% in average for Dry and Dry-Storms for the hyporheic and surface sediment, respectively) (Gionchetta et al., 2019) may determine the lower effect of the long dry phase on the microbial communities, as suggested above. However, the community composition in the

hyporheic zone was still significantly affected by the long-term drought, thus not totally coinciding with that in the control treatment after rewetting.

The overall findings of this study contribute to our understanding of the streambed microbial communities' responses to future hydrological variability. The risk of potential and irreversible modifications of the intermittent streambed microbial communities' diversity and composition, under the increase climatic and anthropogenic pressures, could have important consequences for the development of several in-stream sediment processes, such as biogeochemical reactions, water quality and nutrients cycles.

CHAPTER 3

"Total, active and functional streambed microbial diversity: comparing prokaryote and eukaryote responses to hydrological constrains"

This Chapter is submitted as:

Gionchetta G., Oliva F., Romaní A.M., Bañeras L. Total, active and functional streambed microbial diversity: comparing prokaryote and eukaryote responses to hydrological constrains. *Manuscript under review.*

OVERVIEW

Microbiota inhabiting the intermittent streambeds mediate several in-stream processes essential for the ecosystem functioning. These microbial communities and associated processes, such as nutrient cycling, are influenced by hydrological fluctuations. Streams discharge reduction caused by the strengthened intermittency and the increasing duration of the dry phase are spreading worldwide in response to global climate changes. Here, the impacts on prokaryote and eukaryote diversity inhabiting the surface and hyporheic streambed were examined in laboratory mesocosms once submitted to five months of desiccation, one-week rewetting and punctual storms interrupting the desiccation period. Specifically, five data matrices have been considered to describe the total (DNA-based), active (RNA-based) and functional (CLPP-based) microbial diversity of both prokaryotic and eukaryotic communities. These matrices were compared in order to identify similar or dissimilar patterns of response to the factors studied, and to elucidate to which extent diversity was explained by the environmental and functional variables, such as water content and extracellular enzyme activities, respectively. Most importantly, among the molecular and functional tools applied no redundant information was found, indicating that a comprehensive understanding of the microbial dynamics under hydrological constrains is better when studied through several and complementary techniques. Among the five diversity matrices considered, the RNA-diversity of prokaryotes was the most responding to all the experimental factors considered, such as treatments, time and sediment depth. Water content and extracellular enzyme activities explained large part of the functional diversity (CLPP) and RNA-diversity for both prokaryotes and eukaryotes. Remarkably, functional diversity of streambed microbiota appeared vulnerable to the hydrological consequences of the global change, and changes in microbial functions could be considered alarming in light of intermittent stream ecosystem conservation.

BACKGROUND

Unique microbial community associations coexist in the complex structure of streambed biofilm covering any inorganic or organic substrates (Lock 1993; Mora-Gómez et al., 2016). Bacteria, algae, fungi, protozoa, and small metazoans are usually found within the streambed biofilm, recognized as a dynamic and active ecotone in retaining, amplifying and transforming organic substances and nutrients (Arce et al., 2019; Battin et al., 2016). In this benthic habitat, microbial metabolisms and intricate microbial food webs partake in the global carbon and nitrogen cycles, mainly through degradation of organic matter and coupled nitrification-denitrification which can result in net gas emissions into the atmosphere

(Mulholland et al., 2008, Battin et al., 2008; Beaulieu et al., 2011; Raymond et al., 2013). Streambed microbes and their functions are key forces for the ecosystem functioning and biogeochemical cycles, but at the same time, they are highly sensitive to environmental stressors that may act at different time and spatial scales. Thus, environmental stressors affecting streambed microbes will ultimately determine ecosystem biodiversity and functioning (Duarte et al., 2017; Shade et al., 2012).

In the case of intermittent streams, switches from flooded to dry phases provoke constant fluctuations of water availability and alternated dominance of terrestrial and aquatic taxa within the streambed microhabitats (Febria et al., 2012; Gao et al., 2005; Tamames et al., 2010). Consequently, the specific sensitivity of each population to the hydrological conditions would determine waves of microbial functions and covariation of biodiversity (Romaní et al., 2017). Current climate trends could alter the regular fluctuation of these processes because of the increased environmental changes, such as enlarged duration of drought episodes and abrupt intense rainfalls, affecting temporary streams (Humphries and Baldwin, 2003; Jiménez Cisneros et al., 2014). In extreme streambed conditions, when water disappears for months or water abruptly returns, the prokaryotic community usually revealed high sensitivity in terms of functions which could be coupled to changes in its structure (Marxsen et al., 2010; Zoppini et al., 2014). Two main effects have been proposed to explain the uncoupled relationship between microbial diversity and functioning in the ecosystem. On the one hand, functional plasticity is defined as the capacity of microbial communities to accommodate environmental changes by adjusting their performance (e.g. modifying their organic matter use capabilities to available resources in the environment). On the other hand, functional redundancy, i.e. the co-occurrence of different species with similar roles than can substitute one another, will result in a limited effect on ecosystem function albeit effects on community structure may be relevant (Battin et al., 2016).

Responses of the prokaryotic communities cannot be isolated from changes and interactions with eukaryotes, which may modulate microbial functioning. For instance, locomotion, extraction, scraping, and filtration activity of stream meiofauna, such as protists and metazoans, can affect the microbial communities by changing the fine particles distribution and consequently leading to the formation of new microhabitats (Kathol et al., 2011; Weitere et al., 2018). Also, the grazing pressure by protozoans and metazoans can determine morphological changes in the bacterial community, enhancing their resistance to grazing (Hahn, 2002) and may also promote bacterial diversity (Matz and Kjelleberg, 2005). It is further described that protozoa have the capacity to resist harsh conditions such as anoxia in drying pools (Risse-Buhl et al., 2014). On the other hand, fungi are sensitive to drought but more

resistant than prokaryotes, being able to colonize nearby terrestrial habitats (Romaní et al., 2006a; Wey et al., 2012). (Risse-Buhl et al., 2014). Despite these evidences, the role of eukaryotes in temporary streambed microbial communities is still poorly considered and understood in comparison to that of prokaryotes (Weitere et al., 2018).

Overall, the high complexity of potential relationships within microbial communities demands for the use of complementary methods in order to sensitively test the effect of stressors in the structure and function of streambed biofilms. Among molecular methods, DNA metrics capture the total microbial community (active, dormant and recently deceased taxa) while RNA-based approaches tend to target the active members of the community. Combination of DNA and RNA based methods are useful to determine not only the seedbank of taxa present in the communities but also the subset of those metabolically active (Steven et al., 2017). Despite these recognized assumptions, recent studies showed that calculation and interpretation of activity based on rRNA data should be done with caution, especially in the context of environmental samples (Dlott et al., 2015; Steven et al., 2017). Briefly, an increase of the ribosome content has been related to both the entrance of cells in a dormant state as a life surviving strategy, and to a rapid shift in metabolic functions in order to cope with environmental fluctuations (Flardh et al., 1992; Blazewicz et al., 2013).

However, the information brought by molecular approaches (DNA and RNA-based) may not reflect process rates, such as nutrient cycling or organic matter decomposition, which may need additional measurements (e.g. extracellular enzyme activities, community respiration, functional fingerprint). The analysis of specific enzymes for the degradation of organic compounds can provide specific information about the potential organic matter degradation capacity (Romaní et al., 2012). Among the functional metrics, the practical and useful approach of measuring carbon substrate utilization profiles (e.g. the application of Biolog Ecoplates) informs us about the functional diversity and the metabolic fingerprint, usually named as the community level physiological profile, CLPP (Campbell et al., 2003). Weak or absent correlations between the above functional approaches have been usually observed in the sediment of freshwater ecosystems or in epilitic biofilms (Freixa et al., 2016; Ylla et al., 2012) further indicating that distinct functional metrics may give different, but probably complementary, information. Apparently, the extracellular enzyme activities are more indicative of bacterial activity in the environment, while the CLPP provides information on potential functional diversity (Floch et al., 2011; Freixa et al., 2016).

Extrapolating all the previous information to a methodological context, the combined approach of different molecular and functional toolboxes could better reflect the whole microbial community dynamics occurring in streambed systems under environmental stresses

thus improving description of the microbial dynamics (Langenheder and Lindström, 2019). Accordingly, the main aim here was to stand out similarities or differences between microbial diversity methodological approaches to the imposed hydrological constrains (such as 5 months of dry phase, storms and rewetting simulations) and their relationships to functional responses in a streambed biofilm. In detail, we evaluated: i) the effects caused by experimental factors, i.e. hydrological treatments, time and sediment depth, to the distinct diversity matrices considered; ii) the relationships between the DNA and RNA based microbial diversity and functional (CLPP) metrics calculated for the eukaryotic and prokaryotic streambed components; and iii) to which extent the diversity variability was explained by the environmental and functional variables such as water content and extracellular enzyme activities, respectively.

Separation between total (DNA) and potentially active (RNA) diversity pools were expected, especially under extreme hydrological conditions where DNA matrices were supposed to be more conservative while RNA ones more sensitive to changing environmental conditions. However, correlations between RNA matrices and functional fingerprint as well as between prokaryotic and eukaryotic diversity matrices are suggested because of the activity-mediated metrics and the potential interaction between the two community dynamics, respectively. Water content was supposed an important modulator of the entire community's response. On the other hand, RNA-based prokaryotic matrix was expected to be more correlated to the bacterial-driven functions (e.g. hydrolytic degradation activities) while the eukaryotic matrix to the recalcitrant compounds' degradation.

MATERIALS AND METHODS

Experimental design & sample collection

The experimental set-up was the same as that reported in Chapters 1 and 2. In summary, the long-term drought experiment was performed in 12 streambed sediment columns randomly assigned to three treatments (n=4 replicates per treatment): Control (C), Dry (D), and Dry-Storms (DS). Streambed sediments were obtained from the "Santa Llúcia de Puigmal" stream, a headwater tributary of the Mediterranean river Fluvià (42.217637N, 2.401402E). Sampling was performed on the 4th of February 2016. Columns were prepared on site immediately after sediment collection and each column consisted in a dark plastic cylinder (25 cm height × 15 cm diameter) perforated at the bottom with small holes made with a sterile surgical needle (3 mm diameter), to allow water movement through the sediment column. At 22 cm depth from the surface and in opposite sides of the column two hermetically closed sampling ports (ca. 1 cm^2) were created and used for the sampling of hyporheic sediment.

Each column was placed inside a larger plastic carboy (30 cm height x 25 cm diameter), filled with filter-sterilized (0.2 µm pore size diameter nylon filter, Whatman, Kent, UK) water from the same stream (3L to each column) to ensure water saturation. Water saturation conditions were maintained for all three treatments for a period of 10 days (acclimation period) before applying experimental treatments.

Briefly, the control treatment (C) consisted in sediment columns maintained in saturated conditions with 4 cm of a water layer at the top of each column. Water was renewed twice a week. The dry treatment (D) columns were left to dry, external carboys were emptied and no extra water was added for the next 150 days. For the dry-storms (DS) treatment, columns were also left to dry as in D treatment, but at days 90 and 130, 750 mL of stream diluted water (1:1, filtered stream water: distilled water) were added during 30 min (simulating a summer storm in the area according to dataset 2005-2015 ACA – "Agència Catalana de l'Aigua"). At day 150, all sediment columns were rewetted and maintained under saturated conditions with filtered stream water renewed every two days (Fig. 1).

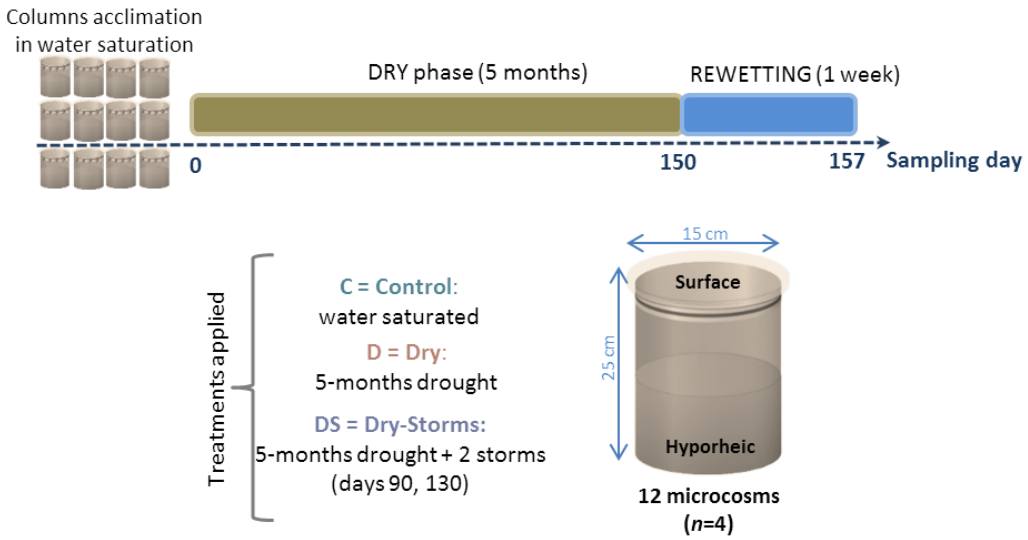


Figure 1. Experimental design adapted to the study.

Rewetting was performed by filling the outside carboys in order to simulate water table resumption with the filtration of water from the bottom of the columns and, at the same time, part of the water was poured into top of the columns to simulate precipitation. All microcosms were placed in incubators (SCLAB, PGA 500) under controlled temperature and light conditions. Light conditions for the entire experiment were set at 12h/12h night/day, exposing the microcosms to a light irradiance (photosynthetic active radiation) of 150 $\mu Em^{-2} s^{-1}$ during

the day and darkness during the night. The range of temperatures applied during the experiment was set according to average values during the spring-summer period recorded in the watershed where sediment communities were collected (data 2005-2015 Idescat https://www.idescat.cat/). Specifically, the day temperature range simulated the passage from late spring (25-29ºC over 6 weeks) to summer (30-31ºC for 12 weeks), while the night temperature was set 4 degrees below the maximum day value (Gionchetta et al., 2019).

Surface and hyporheic sediments were sampled following three crucial experimental moments: after the acclimation period (T0), end of long-term drought (T150) and end of rewetting (T157, Fig. 1). Sediment samples for carbon substrate utilization profiles, extracellular enzyme activities and water content assessment were collected and analyzed on the same sampling day. Sediment samples for nucleic acids extraction were stored at -80ºC until analyzed. Water content (WC) was calculated as the percentage of water loss (%) obtained by the difference between fresh and dry weight.

DNA and RNA extraction

Nucleic acids were extracted from 2 g of sediment with the RNA PowerSoil® Total RNA Isolation Kit (MO BIO Laboratories, Inc.), following the instructions provided by the manufacturer. Purity and concentrations of DNA and RNA extracts were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific; Wilmington, DE, USA). Co-extracted DNA was cleaned up from RNA extracts by using the DNase digestion and RNA Cleanup protocols of RNeasy Mini Kit (QIAGEN, Germany). Later, cDNA was synthesized with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA) according to the manufacturer's instructions. 10 µL of digested RNA extracts at a minimum concentration of 5 ng/µL were used in all cases. Suitability of DNA and cDNA extracts for downstream molecular application was checked after PCR detection of 16S rRNA using universal bacterial primers 357F and 907R.

Microbial community structure determination

The hypervariable V4 region of the 16S rRNA gene for both DNA and cDNA samples was amplified using primer pairs 515F-806R (including bacteria and archaea) and Euk_1391f-EukBr (Eukarya) and methods described by (Kozich et al., 2013) adapted to produce a dual-indexed Illumina compatible library in a single PCR step (Kozich et al., 2013). Primary PCR was performed using fusion primers with target-specific portions (Stoeck et al., 2010), and Fluidigm CS oligos at their 5' ends. Secondary, PCR targeting the CS oligos was used to add sequences necessary for Illumina sequencing and unique indexes. PCR products were normalized using Invitrogen SequalPrep DNA normalization plates. The pooled samples were sequenced using an Illumina MiSeq flow cell (v2) using a 500-cycle reagent kit (2x250bp paired-end reads).

Sequencing was done at the RTSF Core facilities at the Michigan State University USA (https://rtsf.natsci.msu.edu/).

Paired-end sequences were merged, quality filtered and clustered into OTUs (Operational Taxonomic Units) using USEARCH v9.1.13 (Edgar and Flyvbjerg, 2015). Sequences were filtered for minimum length (>250 nt for prokaryotes, and >150 for eukaryotes) and maximum expected errors (<0.25). OTUs were clustered at the 97% identity using UCLUST and checked for the presence of chimeras. OTUs containing only one sequence (singletons) were removed. The subsequent analyses were performed with Qiime v1.9.1 (Caporaso et al., 2011). Representative OTUs sequences were aligned using PyNAST with default parameters against Silva 128 release. The same reference database was used for taxonomic classification of representative sequences using UCLUST.

Sequencing depth ranged between 21,500 and 86,200 sequences of DNA or RNA per sample after removing non-bacterial, unspecific reads. For community analysis, the number of sequences in each sample was normalized by randomly selecting a subset of 21,000 sequences from each sample to standardize sequencing efforts across samples and to minimize bias due to different number of total sequences. DNA and RNA based matrices for prokaryotes (DNA_PRO; RNA_PRO) and eukaryotes (DNA_EUK; RNA_EUK) were created from tables randomized OTU tables keeping only those OTUs that occurred at frequencies higher than 0.2 % of sequences (~100 seqs) in any of the samples contained in the sampling set. OTU tables were curated for low abundance OTUs in order to focus the analysis to the quantitatively important members of the microbial community. Therefore, OTU tables were reduced to 619, 583, 477 and 279 representative sequences for DNA_PRO, RNA_PRO, DNA_EUK, RNA_EUK, respectively. The raw sequence data set (144 libraries) was deposited in the NCBI Sequence Read Archive (SRA) database under accession nº PRJNA546478.

Community level physiological profiles (CLPPs) – functional diversity

CLPPs were obtained from carbon substrate utilization profiles in sediment samples measured with Biolog Ecoplates^{TM} microplates (Biolog Inc., Hayward, CA, USA) in order to determine the differences in heterotrophic metabolic fingerprint (based on carbon source utilization) depending on the treatment, time and depth. Microplates contain three replicate wells of 31 carbon sources assigned to chemical groups as polymers, carbohydrates, carboxylic acids, amino acids, phenolic compounds and amines (Choi and Dobbs, 1999). For each sediment sample replicate we prepared sediment suspensions by extracting and diluting bacteria. Briefly, biofilm was detached from sediment particles by two rounds sonication (1 minute in cold in ultrasound bath, Selecta) and vortex (30 s at maximum speed) of suspensions containing 1 mL of sediment (approx. 1 g of sediment) in 5 mL of pyrophosphate and then 1 ml

diluted in 9 mL of filtered and sterilized stream water. Microplates' wells were inoculated under sterile conditions with 130 µl of diluted sediment extracts and incubated at 20ºC, in dark conditions for 7 days. The optical density (OD) in the plates was recorded at 590 nm every 24 h using a microplate reader (Tecan, infinite M200 Pro).

After 7 days (168 h) most wells had achieved sigmoid color development saturation and the AWCD (Average Well Color Development) was close to 2 (Freixa et al., 2015). Raw absorbance data were corrected by the mean absorbance of the blank wells (3 wells with no substrate) in each plate and values <0.05 were set to zero. Carbon substrate consumption recorded at the end of the incubation (mean of four replicates) for each treatment and sediment depth standardized by AWCD in each plate was used to generate a substrate utilization profile matrix.

Extracellular Enzyme Activity

The extracellular potential enzyme activities (EEA) of β-D-glucosidase (EC 3.2.1.21, GLU), β-D-xylosidase (EC 3.2.1.37, XYL) and phenol oxidase (EC 1.14.18.1, PHE) were measured as indicators of the capacity to decompose cellulose, hemicellulose and lignin-like compounds, respectively. Fresh sediment subsamples 1 mL (approx. 1 g of sediment) were analyzed 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), and for the oxidative enzyme phenol oxidase (EC 1.14.18.1, PHE), as indicators of the capacity to decompose cellulose, hemicellulose and lignin compounds, respectively. For the extracellular enzyme activities, we followed the procedure presented in Gionchetta et al. (2018). The hydrolytic enzymes, GLU and XYL, values were expressed as nmol MUF gDW-1 h-1 and for PHE enzyme, values were expressed as nmol 2,3-dihydroindole-5,6-quinone-2-carboxilate (DIQC) gDW-1 h-1.

Data analyses

DNA and RNA matrices represent genomic composition and active transcription, respectively, of both prokaryotes and eukaryotes signatures. DNA and RNA matrices were log transformed prior to analyses. CLPP matrix represents substrate use potential and it was obtained through BIOLOG and normalized previous analysis. Transformed data were used to calculate distance matrices between samples, applying the Bray-Curtis similarity index for DNA and RNA data, and the Euclidean distance for BIOLOG data. Water content (WC) and extracellular enzyme activities (EEAs) variables, which represent the resources acquisition, were fourth root transformed. For each distance matrix, in order to evaluate and test the effects of the three experimental factors considered (i.e. treatment (TR), time (TI) and sediment depth (DE)), a 3-way factorial permutational MANOVA (PERMANOVA) was performed, testing the main effects and the double interactions. Mantel-like tests were

performed to evaluate whether significant relationships between the five distances matrices considered were present (RELATE test with Spearman's rank correlation). The test assessed the significance of the global correlation, obtained with all data (all experimental conditions). To find the best match between the explanatory variables water content (WC) and extracellular enzyme activities (GLU, XYL and PHE) with the multivariate patterns of the biotic assemblages (DNA and RNA of eukaryote and prokaryote communities), all the possible models were run using the BEST analysis (Method BIOENV, Spearman's rank correlation) followed by the application of distance-based Linear Model and the associated distance-based RDA (Dist-LM). Significance level was set to $\alpha=0.05$ for all tests, but values close to the threshold ($0.05 \leq p < 0.1$) were considered. All the statistical analyses were assessed with PRIMER 7 PERMANOVA+ (Primer-E Ltd., Devon, UK). As supplementary material (Table S1), the communities' composition (main groups and phyla relative abundance) for prokaryotes and eukaryotes are reported.

OUTCOMES & DISCUSSION

The two main experimental factors, drought treatments and sediment depth, determined strong variations in the water content (Table 1). Water content was increasingly reduced in the surface of the mesocosms after 150 days of incubation in response to the applied treatments: Control, Dry-Storms and Dry. A similar trend was observed for the hyporheic sediments although Dry-Storms treatment still maintained some humidity (5.5% WC) (Table 1). Humidity in all treatments and sediment layers resumed to relatively high WC values (~22%) for all treatments and sediment layers after the rewetting phase. As expected, oxygen concentration was specially reduced in the hyporheic zone of the Control treatment (water saturated) and anoxic conditions developed. Dry and Dry-Storms treatments sediments remained well oxygenated all through the experimental time (Table 1).

Table 1. Values of water content (%±SD) and the average dissolved oxygen (D. Oxygen in mg/L±SD) in the sediments monitored during the experimental for each treatment and sediment depth. The experimental time is indicated with T0, T150 and T157 whereas the three treatments are indicated as C: Control, D: Dry, DS: Dry-Storms.

		water content T0		water content T150		water content T157		D. Oxygen	
Depth	Treatment	%	± SD	%	± SD	%	± SD	mg/L	± SD
SURFACE	*C*	17.27	2.19	29.25	1.29	23.86	4.43	4.07	1.68
	D	15.58	1.55	0.44	0.91	22.71	4.43	8.54	0.46
	DS	16.57	1.26	2.73	0.91	21.58	4.43	8.71	0.55
HYPORHEIC	*C*	43.29	1.30	27.45	1.21	23.73	2.68	0.07	0.04
	D	44.71	1.06	0.65	1.05	22.06	2.68	8.50	0.61
	DS	43.43	1.31	5.48	1.05	20.31	2.68	8.31	1.46

Sensitivity of the distinct diversity matrices to the experimental factors

As initially hypothesized, the prokaryotic RNA-based dissimilarity matrix was much more sensitive to the experimental factors studied compared to that of the DNA-based. The first two axes of both DNA and RNA based principal coordinated analysis (PCO) explained about the 64% of the total variation, but significant responses according to experimental factors were found only for RNA data (Table 2, Figs. S1A and S1B). Specifically, the PERMANOVA results showed that the RNA-prokaryote community was mainly responding to the Treatment factor (and Treatment X Time) and secondarily to the depth of the sediment (Table 2, Fig. S1B). The significant response of the RNA-based prokaryotic diversity to the strong changes in water content (treatment and time) suggests the sensitivity of the active fraction of this community to desiccation and probably their rapid adaptation to these changes. Previous studies have reported that water stress and subsequent sediment flooding usually end up with the variation in prokaryotic diversity and activity due to the changed resources availability and water content (Fazi et al., 2013; Romaní et al., 2017; Sabater et al., 2016; Ylla et al., 2010). Changes in active fraction of the prokaryotic community may be further due to distinct strategies to cope with dryness: on the one hand those species capable to resist (for example those that invest energy for the production of substances which increase their protection from dryness such as extracellular polymeric substances, Gionchetta et al., 2018) will be represented; on the other hand those species that become “no-active” or enter into a dormant state will be under-represented. The effect of depth on RNA-based diversity might be

also linked to a survival strategy to drought such as active or passive downwards migration to the hyporheic zones as the water table recedes due to desiccation of the upper parts. In contrast, the DNA-based prokaryotic diversity might include DNA from active and non-active populations, including those that are not responding to the desiccation effect, and could explain the lack of a significant effect of the experimental treatments on DNA-based diversity.

In the case of eukaryotic community, RNA and DNA metrics were equally sensitive but showed significance to different factors, such as the sediment depth and time, respectively (Figs. S1C and S1D). The eukaryote RNA diversity ordination explained about 37.5% of the total variation and it showed higher sensitivity to the depth factor compared to that of time or treatment (Table 2, Fig. S1D).

Hyporheic eukaryotic communities tended to be more similar across time than surface eukaryotic communities regardless of treatment (Fig. S1D). Eukaryotes are known to be able to cope with water stress or rewetting episodes (Cornut et al., 2010; Manzoni et al., 2014; Mora-Gómez et al., 2016; Unger et al., 2009), although the oxygen level of the sediment and water content could influence their survival and activity, forcing their migration from the surface to the deeper and moister hyporheic zone (Norf and Weitere, 2010; Risse-Buhl et al., 2014). Oxygen content of the sediment was occasionally monitored and it was generally lower on the Control treatment compared to Dry and Dry-Storms treatments (Table 1). As expected, oxygen concentrations tended to unify along the sediment column in the Dry and Dry-Storms treatments during the drying process. On the contrary, anoxic conditions developed in the hyporheic area of Control treatments immediately after the acclimation time. The lower dispersion observed among the hyporheic treatments compared to those at the surface suggested that the combination between oxygen content and moisture of the sediment potentially favored the maintenance of fairly stable eukaryotic community, which was confirmed by the relative abundance of major taxa (Fig. S1D and Fig. S2). On the other hand, the ordination analysis of the DNA-based eukaryotic diversity explained about 46.9% of the total variation by the first two axes, and reflected a clear separation across the experimental time (Fig. S1C).

Table 2. Results from PERMANOVA analyses for each diversity matrix (for prokaryotes: DNA_PRO and RNA_PRO; for eukaryotes: DNA_EUK and RNA_EUK; for functional diversity: CLPP) and experimental factors (i.e. Time; Depth; Treatment and their interactions). Significant p-values ($p<0.05$) are indicated in bold whereas in italic when at the limit of significance ($p<0.1$). Not significant results are indicated with 'n.s.'.

	DNA_PRO	RNA_PRO	DNA_EUK	RNA_EUK	CLPP
Time	n.s.	**0.007**	**0.018**	n.s.	**0.001**
Depth	n.s.	**0.045**	n.s.	**0.004**	**0.015**
Treatment	n.s.	**0.011**	*0.082*	*0.089*	n.s.
Time x Depth	n.s.	n.s.	n.s.	n.s.	**0.011**
Time x Treatment	n.s.	**0.016**	n.s.	n.s.	n.s.
Treatment x Depth	n.s.	n.s.	n.s.	n.s.	n.s.

Community Level Physiological Profiles (CLPPs) showed a high sensitivity to the experimental time, sediment depth and to their interaction (Fig. S1E). A clear separation in the carbon substrates utilization across time explained the 54.3% of the total variation and suggested changes in the microbial metabolic functions (mainly bacteria-driven activities) (Fig. S1E). Specifically, the three experimental moments (initial, end of the dry phase, and rewetting) were characterized by different activities of carbon substrates utilization, supporting the idea of a potential specialization of the microbial community functions caused by the alternation between wet-dry-rewet conditions (Fig. S1E). Other studies have previously underlined the sensitivity of the CLPPs method for the detection of shifts in the microbial functional diversity when environmental changes were influencing the streambed microbial community (Freixa et al., 2016; Li et al., 2018; Romaní et al., 2012). Although it was not measured in this study, changes in the quality (biodegradability) and the availability of organic matter may have a role in the observed changes of the functional fingerprint. Indeed, no external inputs of organic matter have been added during the experiment, thus most probably the organic matter quality changed leading to a progressive accumulation of complex recalcitrant compounds during the dry phase (Romaní et al., 2006b; Ylla et al., 2010). After the rewetting, the presence of water and the liberation of previously occluded labile compounds may have favored the selection of different substrates (Romaní et al., 2013; Shumilova et al., 2019).

Relationships between DNA, RNA and CLPP matrices

Few statistically significant correlations were observed between the five dissimilarity matrices (Table 3). Nevertheless, DNA-eukaryote and DNA-prokaryote matrices were significantly correlated ($p = 0.002$, Table 3) suggesting that the total eukaryotic and prokaryotic

communities may experience similar composition shifts under the hydrological constrains simulated.

Table 3. Results from Mantel-like test (RELATE matrices comparison) with Spearman's rank correlation and p-value (in parenthesis), indicated in bold when significant ($p<0.05$) whereas in italic when at the limit of significance ($p<0.1$). Each matrix is identified as: for prokaryotes, DNA_PRO and RNA_PRO; for eukaryotes, DNA_EUK and RNA_EUK; for functional diversity, CLPP.

	CLPP	**DNA_PRO**	**DNA_EUK**	**RNA_PRO**	**RNA_EUK**
CLPP	1				
DNA_PRO	-0.267 (0.941)	1			
DNA_EUK	-0.303 (0.982)	**0.630 (0.002)**	1		
RNA_PRO	-0.046 (0.566)	*0.316 (0.066)*	0.206 (0.152)	1	
RNA_EUK	0.065 (0.197)	-0.035 (0.625)	-0.055 (0.733)	-0.010 (0.508)	1

As inhabitants of the aquatic and terrestrial interface, the microbiota of the sediment biofilm coexists under intricate food-web interactions (Romaní et al., 2017), thus the strength of their interconnection could explain the relationships observed between the prokaryotic and eukaryotic communities based on DNA. Although the effect of the experimental treatments to both matrices were almost inexistent (excepting the effect of time on Eukaryotic-DNA diversity), this correlation may indicate that changes in prokaryotic DNA pool covariate with changes in the eukaryotic DNA pool underlining the connection between both communities (e.g. belong to the same biofilm association). On the other hand, the lack of correlation between RNA-prokaryote and RNA-eukaryote matrices underlines their differential response to the experimental treatments determining changes in the active community. Under dry streambed conditions, eukaryotes tend to be more resistant (e.g. larger motility, stronger cell membrane) in comparison to prokaryotes (Hahn, 2002; Jürgens et al., 1999; Shade et al., 2012; Simon et al., 2016). Usually, water stress episodes result in an increase of eukaryote grazing pressure and the consequent activation of grazing resistance strategies of prokaryotes (Jousset, 2012; Matz and Kjelleberg, 2005; Weitere et al., 2018). The grazing resistance (e.g. cell elongation, size reduction, micro-colony formation, production of virulent factors) is strongly dependent to environmental conditions and to specific bacterial traits (Matz et al., 2005; Seiler et al., 2017; Weitere et al., 2018).

Regarding prokaryotes, the DNA and RNA matrices reported a positive correlation, although it was at the limit of the significance ($p = 0.066$, Table 3). Independently from the distinct community metabolic states, DNA and RNA matrices were describing a similar global pattern of the prokaryote responses. This correlation reinforced the idea that the RNA-community characterization (e.g. potentially active cells) may consist in a subsample of the total approached with the DNA metric (Blazewicz et al., 2013).

In contrast to what expected, the RNA based prokaryotic diversity was not correlated to the microbial community functional fingerprint ($p>0.05$, Table 3). Certain limitations of the community level physiological profiles (CLPPs) method used (Biolog Ecoplates) could be responsible for this. Drawbacks of the CLPPs technique mainly rely in being a culture-dependent method, reflecting only a portion of the whole community together with the toxic effect that the tetrazolium redox dye could have on some species (Campbell et al., 2003; Konopka et al., 1998; Muńiz et al., 2014). Furthermore, although the large spectrum of resources offered by the carbon utilization profiles, including ecologically relevant carbon substrates belonging to distinct guilds (e.g. carbohydrates, polymers…), the definition of the CLPPs is limited to the 31 specific substrata included in the microplate. Even though, the obtained results suggest that RNA and CLPPs may not reflect the same response to the studied factors since while RNA community was more responsive to the drought treatments, CLPPs were more responsive to depth. As a consequence, bacteria belonging to different species would express similar functional fingerprints but harbor essentially different rRNA signatures. Previous studies on streambed microbiota influenced by drying stress reported cases of functional redundancy, observing that changed CLPP not necessarily implied modification of the microbiota total or active diversity (Foulquier et al., 2015; Frossard et al., 2012; Martiny et al., 2013). Therefore, as recently observed, when functional redundancy and/or diversity are high, the microbial community resilience would be more easily conserved when facing environmental fluctuations (Zeglin, 2015).

Diversity variability explained by the environmental and functional variables

Water content was significantly related to all the diversity matrices considered (at the limit of the significance for the DNA-based prokaryotic diversity, Table 4). The distance based linear models revealed that among the environmental (water sediment content) and functional (extracellular enzyme activities) variables considered, the water content of the streambed consisted in the most important driving force describing the diversity variability (Table 4). The DNA reflected the legacy of the treatments because inactive taxa were included, whereas the RNA expression was presumably much more responsive to the changing conditions.

Table 4. Results from BEST (Biota and/or Environment matching) and results from Distance-based linear models (Dist-LM) for each matrix: A) DNA prokaryote, B) RNA prokaryote, C) DNA eukaryote, D) RNA eukaryote, E) CLPPs. The environmental variables considered are indicated as: WC, water content; PHE, phenol-oxidase; XYL, β-xylosidase; GLU, β-glucosidase. Significant p-values ($p<0.05$) are indicated in bold whereas in italic when at the limit of significance ($p<0.1$).

A) DNA_PROKARYOTE

BEST matching and selection			Dist-LM		
Correlation	Variable Selection	Best Rho (Sign.)	Sequential	Sign.	R^2
0.305	WC	0.305 (0.222)	WC	*0.091*	0.25
0.298	PHE + WC		PHE	0.317	
0.245	XYL + PHE + WC		XYL	0.803	
0.133	GLU+ XYL+ PHE+ WC		GLU	0.667	

B) RNA_PROKARYOTE

BEST matching and selection			Dist-LM		
Correlation	Variable Selection	Best Rho (Sign.)	Sequential	Sign.	R^2
0.458	GLU	0.521 (**0.016**)	WC	*0.054*	0.47
0.501	GLU+XYL		PHE	**0.026**	
0.516	GLU+XYL+PHE		XYL	0.349	
0.521	GLU+XYL+PHE+WC		GLU	**0.037**	

c) DNA_EUKARYOTE

BEST matching and selection			Dist-LM		
Correlation	Variable Selection	Best Rho (Sign.)	Sequential	Sign.	R^2
0.353	WC	0.353 (0.130)	WC	**0.015**	0.31
0.283	XYL+WC		PHE	0.410	
0.201	GLU+XYL+WC		XYL	0.744	
0.126	GLU+XYL+PHE+WC		GLU	0.262	

D) RNA_EUKARYOTE

BEST matching and selection			Dist-LM		
Correlation	Variable Selection	Best Rho (Sign.)	Sequential	Sign.	R^2
0.061	GLU	0.120 (0.322)	WC	*0.056*	0.31
0.120	PHE+WC		PHE	0.219	
0.088	GLU+PHE+WC		XYL	0.456	
0.065	GLU+XYL+PHE+WC		GLU	0.184	

E) CLPP

BEST matching and selection			Dist-LM		
Correlation	Variable Selection	Best Rho (Sign.)	Sequential	Sign.	R^2
0.116	PHE	0.180 (0.449)	WC	0.126	0.42
0.174	GLU+XYL		PHE	*0.083*	
0.180	GLU+XYL+PHE		XYL	**0.004**	
0.071	GLU+XYL+PHE+WC		GLU	0.406	

So far, limited loss of water content from the streambed submitted to a prolonged dry phase showed important consequences, such as the conservation of availability and quality of organic compounds as well as the reduction of the cells water stress (Gómez et al., 2012; Moyano et al., 2012). Our findings stood out that sediment moisture molded the diversity patterns independently from the metabolic status of the eukaryotic and prokaryotic microbial communities, giving equal importance to the DNA and RNA-based diversity metrics. Only the RNA-based prokaryotic diversity showed strong relation with all the environmental and functional variables considered (Spearman correlation: 0.521, p = 0.016, Table 4). Specifically, the first two axis of the distance-based RDA explained the 83.0% variation out of fitted model and the 39.1% out of total variation, where the water content (p = 0.054) and extracellular β-glucosidase (p = 0.037) and phenol-oxidase (p = 0.026) activities were significantly correlated to the prokaryotic diversity (Fig. 2A, Table 4). In particular, prokaryotes inhabiting the control treatment (C) appeared related to the β-glucosidase function whereas the prokaryotes inhabiting the dry treatments (D and DS) were significantly linked to the phenol-oxidase utilization, at the end of the dry and rewetting phase (T150 and T157 D and DS, Fig. 2A). These results confirmed our initial hypothesis revealing important correlations between prokaryotic community and the hydrolytic activities, as usually considered bacterial-mediated functions (Ylla et al., 2010). Similarly, previous studies revealed a functions specialization trait, reflected by distinct utilization of extracellular enzymes, under dry-wet alternation (Freixa et al., 2016; Pohlon et al., 2013a), and strengthened the idea that changes in the quality of the available organic matter, induced shifts in the utilization of extracellular enzymes linked to the degradation of labile material in wet condition (as in the C treatment), or to the application of the function related to the degradation of lignin-like compounds under prolonged dry condition (as in the D and DS treatments).

Contrariwise to what was expected, the RNA-based eukaryotic diversity matrix was highly correlated with phenol-oxidase once the sediment was rewetted (Fig. 2B, Spearman correlation: -0.50 with dbRDA1) although it was not a significant relationship (Table 4). Conceivably, this result suggested that eukaryotes used different types of resources during the dry phase, for instance compounds released from dead cells accumulated during the dry period or due to the increased grazing pressure. Regarding the functional diversity measured with CLPPs, the first two axis of the distance-based RDA explained the 78.57% variation out of fitted model and the 32.72% of total variation (Fig. 2C, Table 4). The extracellular enzyme activities expression was different depending on the experimental times and reported significant correlation between the wet condition (Time 0) and the β-xylosidase activity (Table 4, Fig. 2C). As opposite, β-glucosidase and phenol-oxidase activities were high among the driest

and rewetted samples, although both at the limit of the significance (Table 4, Fig. 2C). These results confirmed that the hydrological conditions importantly influenced shifts in the utilization of carbon compounds by the microbial communities inhabiting the streambed.

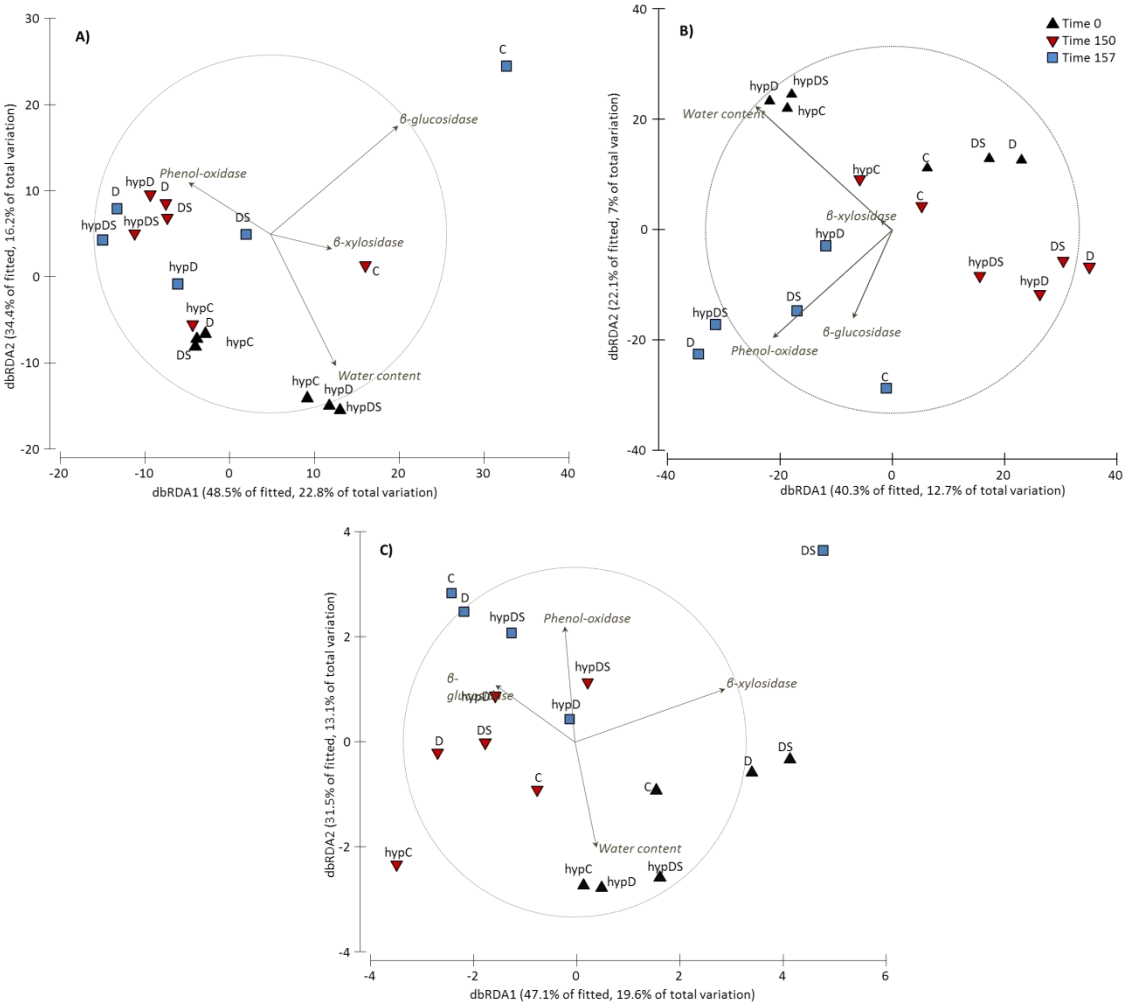


Figure 2. Distance based RDA plots obtained from fitting A) RNA prokaryotes, B) RNA eukaryotes and C) CLPPs, to water content, phenol-oxidase, β-xylosidase and β-glucosidase. Colours indicate the three experimental times: T0; T150; T157. The letters C, D and DS state for the three treatments Control, Dry and Dry-Storms respectively, whereas 'hyp' acronym indicates the hyporheic samples.

CONCLUSIONS

The functional toolboxes, such as the CLPPs and EEAs, confirmed their utility in the description of the ecosystem function under environmental changes, independently from the metabolic state of single cells given by complementary metrics using molecular targets. Our study strengthened the importance of functional metrics (e.g. EEAs) able to reflect potential

functional specialization occurring in the microbial community under altered hydrology, and also underlined the reliability of this method significantly related to the active RNA diversity variability of prokaryote and eukaryote communities. Bearing in mind that the natural complexity cannot be represented through an experimental approximation, each aspect studied here by the distinct diversity matrices (DNA and RNA-based data) appeared complementary and not redundant, reflecting diverse sensitivity to the effects caused by the altered hydrology and explaining a similar amount of the total variability. Our findings underlined that specific microbial dynamics would be better described considering as many functional and molecular tools as possible, since each matrix displayed a distinct sensitivity when the effect of the experimental factors (as experimental time, sediment depth and hydrological treatment) was analyzed.

CHAPTER 4

"Multi-model assessment of hydrological history impact on microbial structural and functional responses in Mediterranean catchments"

This Chapter is submitted as:

Gionchetta G., Artigas J., Arias-Real R., Oliva F., Romaní A.M. Multi-model assessment of hydrological history impact on microbial structural and functional responses in Mediterranean catchments. *Manuscript under review.*

OVERVIEW

Microbes inhabiting dry streambeds manifest adaptations to intermittent flow conditions but the current climate patterns may jeopardize microbial communities' diversity, composition and function, with enlarged duration of the dry period. This study address insights on whether and to what extent the hydrological history affects streambed microbial community diversity (density and diversity indices), composition, and functions (extracellular enzyme activities and respiration). To this aim, a network composed of 37 sites was selected including perennial and intermittent streams in Mediterranean basin (NE Spain). The hydrology was monitored at each site over 8 months, previous to the sampling that was conducted in wet conditions (early autumn). In parallel, several environmental stressors, at the site and watershed scale, were measured and considered for further modelling of microbial community responses.

Our results showed that the hydrological history modulated the community composition and to some extent the activities carried out under different environmental conditions. A step change of the microbial composition was observed, as the driest intermittent communities were got separated from those of the flowing sites. Microbial functional metrics showed a progressive increase of recalcitrant carbon degradation activity at sites with extended dry phase. In contrast, bacterial biomass and microbial diversity were mainly influenced by sites characteristics and catchment land uses. Together, these findings stood out that the ecological risk assessment of dry streambeds needs to consider both land use and hydrological history to ensure fluvial ecosystem functions carried out by sediment microbiota.

BACKGROUND

Dry streambed sediments are dynamic ecotones where terrestrial and aquatic ecosystems interact and their functioning is largely driven by microorganisms hosted and shaped by temporal environmental fluctuations (Arce et al., 2019; Datry et al., 2017). Currently, dry streambeds are spreading worldwide according to the expanded flow intermittency. The reduction of annual precipitation, the increase of water extraction and the global raise of temperature are the major factors intensifying flow intermittency, especially in the Mediterranean areas (Cipriani et al., 2014; Schewe et al., 2014). Most Mediterranean intermittent streams, such as those flowing in the north-eastern part of the Iberian Peninsula, usually cease their flow for about one-to-two months every year during the summer period. At present, it has been reported for intermittent streams to exhibit extended dry-phase episodes also coupled with intense but sporadic precipitations (Duran et al., 2017).

The increase of streambed aridity in intermittent streams and the consecutive reduction of sediment water content due to the extended air-exposition may affect the hosted microbiota

and compromise the microbial metabolism (Febria et al., 2015; Gionchetta et al., 2019; Marxsen et al., 2010; Rees et al., 2006). Microbial streambed communities largely partake in key ecosystem processes such as nutrient cycling and biogeochemical processes, and their diversity and metabolic capabilities are fundamental for the maintenance of fluvial ecosystem functioning (Datry et al., 2017). Studies reported that a long period of water stress reduces specific microbial activities linked to carbon degradation, most probably due to i) the transition of microbes to a dormant stage (Jones and Lennon, 2010; Schimel, J., 2007), ii) the allocation of a large fraction of the cell metabolic costs for developing survival life strategies to cope with desiccation (such as extracellular polymeric substances production) (Frossard et al., 2012; Lake, 2003; Romaní et al., 2017) and/or iii) the reduced organic matter readily available for microbes during the drought condition, as suggested by the expression of specific microbial activities indicating recalcitrant compounds accumulation on the streambed surface (Freixa et al., 2016; Ylla et al., 2010). On the other hand, and similarly to arid soil systems, frequent or sporadic precipitations interrupting dry periods can determine sharp punctual increases of streambed sediment respiration activity resulting from awakening of dormant microbes (Bérard et al., 2011; Evans et al., 2014; Marcé et al., 2019; Meisner et al., 2017).

Streambed microbial assemblages are shown to be also affected by drying-rewetting cycles, especially when experiencing unusual extended dry phases (Meisner et al., 2018; Pohlon et al., 2013a). Specific taxa composition in streambed sediments suffering hydrological alterations may reflect a shift towards a community able to cope with more extreme environmental conditions (long drought and ephemeral-intense rainfalls). This shift may result into a more specialized community including species showing specific life strategies to cope with drought (Evans & Wallenstein, 2014), such as thicker cell wall (e.g. in bacteria and fungi, Jones & Lennon, 2010; Shade *et al.*, 2012), the ability of endospore formation or the increased production of extracellular polymeric substances (EPS) in microbial aggregates (biofilm) inhabiting the dry soil and streambed (Flemming *et al.*, 2016; Gionchetta *et al.*, 2019). For instance, in experimental studies, specific phyla, such as Actinobacteria, Firmicutes and Bacteroidetes, have been described as good competitors during periods of maximum desiccation (Fazi et al., 2008; Klappenbach et al., 2000). In the case of intermittent streams, the 'terrestrialisation' process of the dried stream channel (Arce et al., 2019), where the aquatic vegetation is replaced by terrestrial taxa (Holmes, 1999; Westwood et al., 2006), could be associated to a terrestrial-like transition of the microbial community inhabiting the streambed, acquiring features similar to the surrounding soils. On the other hand, upon rewetting, the osmotic stress initially forces the persistence of resistant phyla (e.g. Firmicutes and Actinobacteria) but later on the microbial communities gradually change to a larger

abundance of wet-favoured taxa, such as Proteobacteria together with Verrucomicrobia, Bacteroidetes and Acidobacteria (Pohlon et al., 2013b; Schimel and Schaeffer, 2012).

In addition to hydrology, streambed microbes in intermittent systems may be further affected by co-occurring environmental factors related to the catchment characteristics acting at different spatial scales. The physical characteristics such as the stream-order and the type of streambed substrata (e.g. cobbles/bedrock, fine/coarse sediment, accumulation of leaf litter) can influence the microbial response to hydrological intermittency (Arce et al., 2019; Duarte et al., 2017). In intermittent rivers, first order headwaters are usually more affected by natural intermittency compared to the downstream, due to their hydro-morphological features (e.g. pronounced slope, distinct geo-morphology reducing the hyporheic zone). Despite first order branches are generally more isolated from the largest mid- and down-stream parts, headwaters have been described as microbial biodiversity reservoirs, reflecting high variability of habitats and resources (different organic matter quantity and quality), and/or large microbial colonization from the adjacent soils than that observed in downstream waters (Besemer et al., 2013). Thus, the effect of intermittency on headwater streambeds is especially sensitive and key to preserve watershed microbial biodiversity. Furthermore, greater accumulation of leaf litter and coarse sediment particles are mostly expected in headwater sections and the mixed fine-coarse sediment granulometry can reduce the loss of organic matter and water content during drying periods limiting the associated decrease of microbial activities (Freixa et al., 2016). In addition, other studies 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 preserve water loss from sediments under dry conditions, was considered as a hydrologic 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 those factors related to the use of the surrounding soils (e.g. agriculture or industrial activities) or to the reduction of the natural riparian vegetation (Allan, 2004; Bruno et al., 2016; Steward et al., 2018, 2012).

Until present, studies on the effect of intermittency on streambed microbes have been mainly focussed on the wet (and rewetting) period and less attention has paid on the drought period, being mostly laboratory experiments, and few field experiments focussing on a single or a limited number of stream ecosystems (Freixa et al., 2016; Timoner et al., 2012; Zeglin, 2015). However, to better understand streambed microbial responses to intermittency, field studies embracing the multiple pressures (including hydrology history and other environmental factors) are needed, as shown by recent studies where interactive and jointly effect of multiple

factors on biological communities in natural ecosystems are shown (Besemer et al., 2013; Bruno et al., 2016; Dickie et al., 2015; Gieswein et al., 2017).

Accordingly, the present study attempted to fill this gap evaluating the functional and structural (in terms of diversity and composition) responses of streambed microbial communities to a natural dryness gradient from intermittent streams located in the Mediterranean basin. Being the duration of dry and wet periods highly unpredictable, the hydrology of each of the 37 selected sites was monitored *in situ* and classed later obtaining a natural gradient of dryness history. From the 8 months of the hydrological monitoring several parameters were calculated, such as the total dry phase duration or the duration of the last wet event before our sampling. In parallel, a range of environmental factors were measured and included as potential predictors for the streambed microbial community responses (e.g. riparian vegetation, organic matter content, land use).

Specifically, this investigation aimed to i) elucidate whether and to which extent the hydrological history was influencing the microbial diversity, composition and activity of sediment microbial communities in the selected Mediterranean intermittent streams; ii) determine the relative contribution of catchment characteristics (riparian vegetation, organic matter and water content, and land use) to modulate the response of microbial diversity, composition and activity in these communities; iii) define patterns of, and linkages between, the bacterial community composition and their metabolic activities along the dryness gradient. In sites with long dry-phases, we expected a decrease of microbial labile carbon degradation capacities but an increase of recalcitrant material utilization accompanied by a transition of microbial community diversity and composition to a soil-like microbial community. Overall, we hypothesized that the microbial functions would be more responsive than diversity, and mainly influenced by the organic matter and water content in sediments considered as major environmental forces influencing microbiota activities.

MATERIALS AND METHODS

Study area, sites selection and sampling strategy

A total of 37 upstream sites were selected and surveyed in streams flowing across the north-eastern part of the Iberian Peninsula (Catalonia). These sites belong to nine watersheds corresponding to the main rivers of that area flowing to the Mediterranean Sea, i.e. Muga, Fluvià, Ter, Tordera, Llobregat, Francolí, Foix, Besós and Ebro rivers (Fig. 1). The sampling sites included lowland and mid-mountain rivers (altitude <1200 m.a.s.l.) which exhibited a substantial variation in mean annual precipitation (350–1200 mm), a seasonal intermittency with increased duration of the dry period, and different combinations of natural, semi-natural

and anthropogenic land-uses (Fig. 1, more information from ACA Agència Catalana de l'Aigua public data). The majority of the sites selected for the study were first order tributaries and characterized by a relatively low anthropogenic land use.

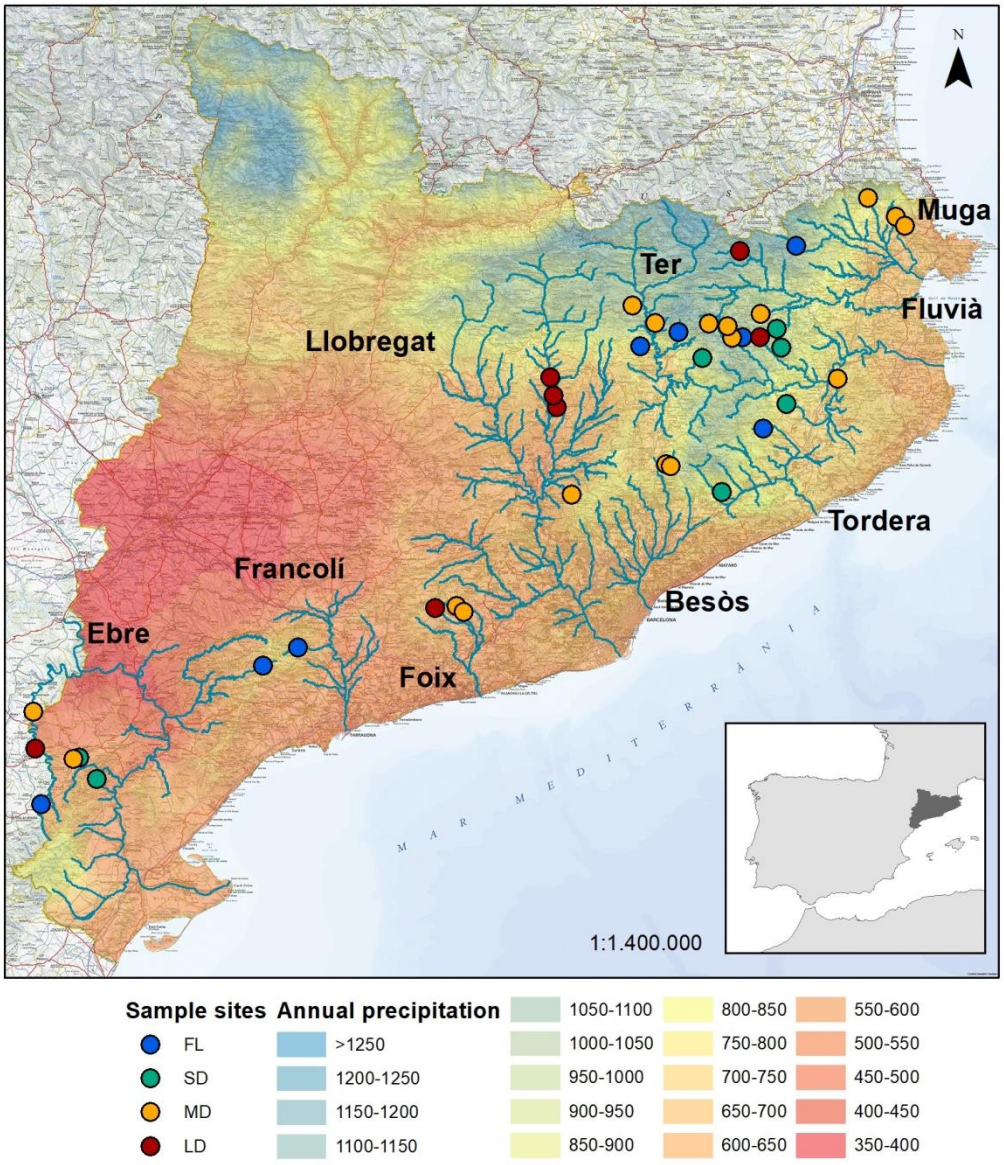


Figure 1. Map showing the streambed sampling sites selected in Catalonia (ArcGIS v.10). The colour gradient represents the values of annual precipitation (www.icc.cat) and the sites colour indicates the different sites groups (FL: Flow; SD: Short Dry phase; MD: Medium Dry phase; LD: Long Dry phase).

The sampling campaign was at the beginning of the wet season in autumn (shortly after the warm summer of 2016), in order to homogenize the hydrological condition among sites before sampling and sample them in wet conditions. The location of the 37 sites and their distance to laboratory forced us to perform the samplings at different days, from the 19th of October to the 23th of November. All sites belonging to the same watershed were sampled in one day,

with the exception of Ter and Ebro rivers which, due to a larger number of sampling sites, were sampled during two days. At each site, five surface sediment sample replicates were collected randomly with a methacrylate corer (5 cm depth and 4.5 cm in diameter) and transported cold (4ºC) to the laboratory. The percentage of streambed shadow cover (e.g. <10% unshaded, 10-50% highly illuminated, 50-80% shaded with light spots, >80% totally shaded, considered length 100 m) and riparian vegetation coverage (e.g. the presence of trees, shrubs or small bushes was considered in percentage, from <25% between 25-50%, 50-75% and >75%, for the first 10 m width of riparian vegetation) were measured *in situ* following a field-assessment procedure.

Once at the laboratory, subsamples of ca. 1 ml sediment volume (1 cm^2 projected surface area) were created and collected from each sediment corer with an uncapped syringe for the analysis of extracellular enzyme activities, respiration, bacterial community composition and density. Community respiration and extracellular enzyme activities were measured within 24h after sampling. Samples for bacterial density were fixed with 10 mL of filter-sterilized (filtered by 0.2 µm pore size nylon filters Whatman, Kent, UK) synthetic water (salts dissolved in Milli-Q water) and formaldehyde (2% final concentration), and then stored at room temperature. For the microbial diversity analysis of 16S rRNA gene the sediments collected were stored at -20ºC. The remaining extra sediment was used for organic matter and water content determinations.

Hydrological history and catchment characteristics of the study sites

One year before the sampling, temperature and pressure transducers (Solinst Levelogger Gold Model 3001 and Solinst Barologger Gold Model 3001, Solinst Ltd, Georgetown, ON, Canada), and temperature data loggers (ACR SmartButton Logger, MicroDAQ) have been installed to characterize the hydrology of the different studied sites. The hydrology of each stream site was extrapolated as daily variation of the streambed temperature corrected for the barometric pressure and air temperature. The daily variation was determined as the difference between the maximum and minimum temperature per day and the daily higher rate of change per hour. The resulting serial number of flow days and dry days allowed us the calculation of the diverse parameters regarding the hydrological history of sites (see Supplementary Information S1, Arias-Real et al. 2018). During the hydrological monitoring programme, we also measured the physical-chemical characteristics of the sites when water flow was present (Supplementary Information S2). The hydrological data collected accounted for 8-months (245 days) of hydrology history for each stream site and the related parameters calculated from this database were: the total number of dry days over the 8-months (T_DRY); the percentage of dry days over the 8-months (P_DRY); the duration (days) of the last dry event previous to the sampling (L_DRY); the duration of last rewetting period (i.e.

the number of consecutive wet days previous to the sampling) (REW); the number of dry/rewetting cycles over the 8-months (FREQ). Apart from hydrological descriptors, *in situ* data corresponding to sediment organic matter, water content, shadow cover and riparian vegetation, and catchment land-use characterization of sites were determined. The organic matter content was measured as AFDW (Ash Free Dry Weight) and one mL of sediment sample was dried at 70°C during 72 h and burnt for 4 h at 450 °C using a muffle furnace (AAF 1100, Carbolite, UK). The results were expressed as grams of AFDW per gram of sediment dry weight (DW). Water sediment content was calculated as percentage of water loss (%) obtained by the difference between fresh and dry weight (1mL for each sample). Major land-uses (urban zones, agriculture and forest areas, Corine Land Cover 2012, https://www.eea.europa.eu/data-and-maps/data/clc-2012-raster) have been calculated as percentage for the whole stream basin and just for the adjacent 1 km upstream site's catchment (i.e. the intersect between the entire catchment and a buffer radius of 1 km centred on the sampling point).

Microbial response variables

Bacterial density and community respiration

Bacterial density in sediments was determined by flow cytometry (FACSCalibur, Becton Dickinson) following a protocol adapted from Amalfitano et al. (2008). Sediment samples were sonicated for 1 min, shook for 30 s, and sonicated again for 1 min to extract the biofilm from 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 cells' separation. Samples were shaken for 30 min (150 rpm, dark and room temperature) and then left 10 min at 4 °C and sonicated with ice for two cycles of 1 min. Later, the samples were shacked again for 1 min and left for 5 min to allow sedimentation of larger particles and finally 1 mL of supernatant was transferred into a clean sterile Eppendorf. Once obtained the sediment extracts, each sample was purified through Nycodenz (1 mL) added at the bottom of each Eppendorf and samples were centrifuged (14 000 rpm) for 90 min at 4 °C. Purified sediment extracts (400 µL) were stained with Syto13 (4 µL Fisher, 5 µM solution) and incubated in the dark for 30 min. Stained samples were counted using flow cytometry (FACSCalibur, Becton Dickinson). To normalize fluorescence data, a known concentration of beads solution (10 µL of 10^6 beads·ml^{-1}, Fisher 1.0 µm) was added to the samples. Results of bacterial density (BAC) were expressed as cells × 10^8 per grams of sediment dry weight (DW).

Resazurin assay was applied for the community respiration (RESP) determination and modified from Haggerty and colleagues (Haggerty et al., 2010). The resazurin (7-Hydroxy-3H-

phenoxazin-3-one-10-oxide sodium salt, RAZ) method consists in using a redox dye (blue in oxidised state) that indicates the respiratory activity of microorganisms (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), shook for 30 s, and sonicated again for 1 min to extract the biofilm from sediment grains (Ultrasons, Selecta). Subsamples of 1 ml of the extract were diluted in 9 ml of filtered water per 0.2 µm (filtered by 0.2 µm pore size nylon filters Whatman, Kent, UK). Aliquots of each sediment extract (166 µL) were placed in a transparent microplate (MicroWell™ Nunc™, ThermoFisher Scientific) and mixed with ringer solution (28 µL) and resazurin stock substrate (6 µL of 0.35 mM Sigma-Aldrich, USA). The plate was closed with a lid, then incubated (target temperature 20ºC) and every cycle started with shaking for 90 seconds (shaking amplitude 3 mm) and wait for 10 seconds before each absorbance reading. The incubation lasted 24 hours and the absorbance was read every 4 minutes (603 nm and 570 nm resazurin and resorufin wavelengths). Subsequently, we use the resorufin (pink compound which indicates the occurred resazurin to resorufin conversion) standard's curve and the absorbance values obtained after 18h incubation in order to define the community respiration as micromoles of resazurin respired per grams of dry weight per hour (µmol RAZ gDW-1 h-1).

Extracellular enzyme activities (EEA)

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), and for the oxidative enzyme phenol oxidase (EC 1.14.18.1, PHE), as indicators of the capacity to decompose cellulose, hemicellulose and lignin compounds, respectively. For the extracellular enzyme activities, we followed the procedure presented in Gionchetta et al. (2019). The hydrolytic enzymes, GLU and XYL, values were expressed as nmol MUF gDW-1 h-1 and for 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 on 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 manufacturer's instructions. The DNA concentration and quality in each sample were measured spectrophotometrically (Nanodrop2000, Thermo Scientific™, Waltham, USA) and further assessed through 1% agarose gel electrophoresis. DNA extracts were stored at -20°C until sequencing analysis at the Roy J. Carver Biotechnology Center (University of Illinois,

IL, US). The diversity of prokaryotes (including bacteria and archaea) was analysed using the primer pair V4_515F/V4_806R61 (Caporaso et al., 2011), targeting the V4 region of the 16S rRNA gene, on Illumina MiSeq technology. We analyzed both bacteria and archaea species in sediments, but the latter was excluded from analysis due to extremely low reads' values.

The IM-TORNADO pipeline version 2.0.3.2 (Jeraldo et al., 2014) was used to generate OTU table and to 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). Rarefied OTU tables were generated by multiple rarefactions on prokaryotes BIOM table using the *phyloseq* package by R software (R version 3.4.1). The rarefactions curves were generated (*rarefy_even_depth* function, *phyloseq* package) and rarefied OTU table was set to the smallest count of reads available for any sample in the table (9211 reads/sample) in order to equalise sequencing depth samples. Two alpha-diversity metrics were calculated from the OTUs table, such as the Shannon-Wiener index (DIV) and Chao1 richness index (RICH) using the *phyloseq* package by R software (R version 3.4.1). The sequence data generated from this study have been uploaded in the Short Read Archive (SRA) of the National Centre for Biotechnology Information (accession number: 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 principal component analysis (PCA) including all analysed parameters (the total number of dry days over the 8-months, T_DRY; the percentage of dry days over the 8-months, P_DRY; the duration (days) of the last dry event previous to the sampling, L_DRY; the number of consecutive wet days previous to the sampling, REW; the frequency of dry/rewetting cycles over the 8-months, FREQ; the water sediment content, WAT_CON; the organic matter content, ORG_MAT; the riparian vegetation coverage, RIP_VEG; the shadow cover, SHAD; the forested land-use, FORE; the agricultural land-use, AGRI; the urban land-use, URBA). Furthermore, although the hydrological history was mainly studied as a continuous factor, for some general analyses it was categorized and the sites being classified as: flowing sites (FL, always flowing), short-dry phase sites (SD, average 7.58% of dry days over the 8-months), medium-dry phase sites (MD, average 52.50% of dry days over the 8-months), and long-dry phase sites (LD, average 91.72% of dry days over the 8-months) (Fig. 1, Table 1). Differences between hydrological categories (FL, SED, MD and LD sites) were analysed for extracellular enzyme activities, community respiration, bacterial density and indices of microbial diversity and richness by one-way ANOVA.

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. Letters indicate the significance obtained from the ANOVA *post-hoc* comparisons (Tukey test).

	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
P_DRY (% dry days)	0.00[a]	±0.00	7.58[a]	±1.90	52.50[b]	±2.76	91.72[b]	±3.52
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 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/g DW)	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)	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

Predictors considered: total dry phase duration (T_DRY, total number of dry days over the 8-months); percentage of dry days over the 8-months (P_DRY); duration of the last dry period previous the sampling (L_DRY); duration of the last wet period previous 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); shadows cover (SHAD); riparian vegetation cover (RIP_VEG); forested land use (FORE); agricultural land use (AGRI); urban land use (URBA).

To analyse the response of microbial functions (extracellular enzymes and respiration) and diversity (diversity indices and bacterial density) to the hydrological and catchment characteristics we followed the cookbook on multiple-stressors analysis using survey data (Feld et al., 2016). Firstly, we run exploratory analyses (GLU, β-glucosidase activity and XYL, β-xylosidase activity were sqrt (square root) transformed; PHE, phenol-oxidase activity; RESP,

community respiration; BAC, bacterial density, DIV, Shannon diversity index; RICH, Chao1 richness index and RIP, riparian vegetation, were log transformed) to reduce skewness where necessary, consequently we standardized and checked the collinearity among the variables (Feld et al., 2016). Secondly, we rank all the predictors (hydrological history parameters (T_DRY, L_DRY, REW, FREQ), sediment water and organic matter (WAT_CON, ORG_MAT), shadow cover (SHAD), riparian vegetation (RIP_VEG), the agricultural (AGRI), forested (FORE) and urban (URBA) land uses) using Random Forest (RF) analysis (package *ForestSRC*, R software; Ishwaran & Kogalur, 2016). The number of trees in the RF was 3000 and that of variables used in each spit was set to three (Feld et al., 2016). Based on the RF results, we used a generalised lineal model (GLM) to create global models to model the response of each variable to single and combine predictors (Feld et al., 2016; Grueber, et al., 2011; Barton et al., 2016). For each model we used a maximum of four predictors selected considering the RFs, the PCA results and the study questions; specifically, including two hydrological predictor terms (T_DRY and REW) and at latest two environmental predictors for each response variable. We quantified the single and combined predictors and their significance through multi-model averaging approach (*MuMIn* R package). This technique ranks all possible models generated (based on model weight < 0.90 and Akakike's Information Criterion AIC, Burnham & Anderson, 2002). All models were validated by visually checking normality and homoscedasticity of their residuals (Feld et al., 2016; Zuur et al., 2009). A part from the importance of model's predictors obtained modelling, we also calculated the variance partitioning for each predictor, using the function *varpar* (package *variancePartition*, R software) to better determine variables responses. The entire analytical procedure explained was conducted in R software (R version 3.5.2), with the significance level set at $p < 0.05$ for all tests.

Differences in microbial community composition among the study sites were shown by a non-metric multidimensional scaling (NMDS) ordination analysis based on Bray-Curtis dissimilarity performed at the OTU taxonomic level. OTUs abundance matrix used for ordination analyses were previously fourth root transformed. A permutation analysis of variance (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 & PERMANOVA+ version 1.0.1). In order to identify the taxonomic classes significantly related to the OTU distribution specific vector fitting analysis have been applied to the NMDS (Legendre and Legendre, 1998). A further one-way ANOVA was applied to determine significant differences between taxonomic classes' relative abundance inhabiting the different hydrological history categories (FL, SD, MD and LD sites). Finally, a Mantel test was performed between the community composition and functional matrices to assess whether they respond

similarly to flow intermittency (RELATE analysis with Spearman rank correlation in Supplementary Information S3, PRIMER 6 version 6.1.11 & PERMANOVA+ version 1.0.1).

OUTCOMES

Sites classification and catchment characteristics

The PCA resulted in a clear distinction between the hydrological and catchment characteristics gradients along the first and second axes, respectively (Fig. 2). Only the factors with correlations coefficient >0.80 (Pearson correlation, r_p) with the first two PCA components (PC1 and PC2) have been selected to define gradients among sites. The first axis accounted for 40% of the total variability, describing the increase of both the total dry duration (T_DRY, r_p: 0.941, Fig. 2) and the percentage of dry phase duration (P_DRY, r_p: 0.941, Fig. 2), and the concurrent decrease (as along the opposite direction of the PC1) of the number of consecutive wet days previous to the sampling (REW, r_p: -0.946, Fig. 2). The variability along the second axis, explaining 17.3% of the total variation, mainly corresponded to the increase of the land forested coverage (FORE, r_p: 0.861, Fig. 2) and to the decrease (as along the opposite direction of the PC2) of the agricultural land-use (AGRI, r_p: -0.888, Fig. 2).

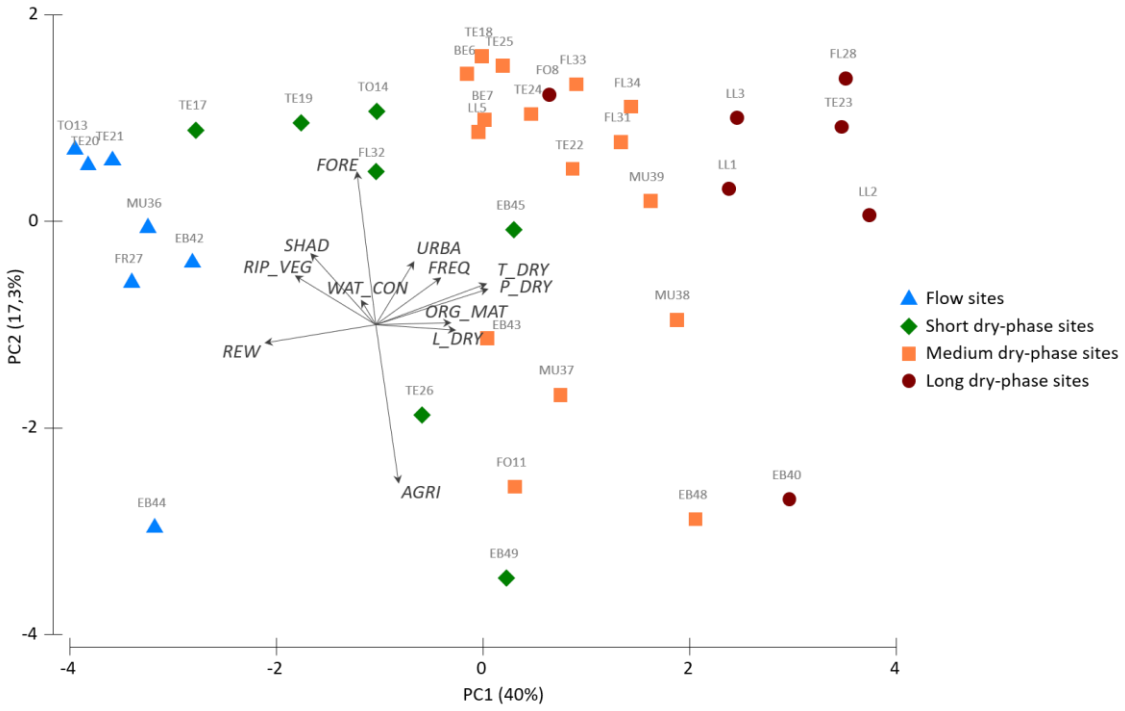


Figure 2. Principal Component Analysis (PCA) showing the importance of the environmental and hydrological that better distinguished our 37 study sites, and represented with the arrows: total dry phase duration over the 8-months (T_DRY); the percentage of dry days over the 8-months (P_DRY); the duration (days) of the last dry event previous to the sampling (L_DRY); the duration of last wet period (REW); frequency of dry/wet cycles (FREQ); organic matter content (ORG_MAT); sediment

water content (WAT_CON); shadow cover (SHAD); riparian vegetation cover (RIP_VEG); agricultural land-use (AGRI); forested land-use (FORE); urban land-use (URBA). The colours and shape of each site define the correspondent hydrological group (legend).

The sites distribution was clearly ordinated along the PC1 according to the four hydrological history categories pre-defined in the methods section (FL, SD, MD and LD sites). The PC2 axis ordinated sites depending on their forested or agricultural land coverage, despite the fact that all selected sites were *a priori* characterized by a relatively low anthropogenic land use (Table 1). Hydrology and catchment characteristics were main drivers shaping the sites distribution, among which long dry phase sites (LD) had significantly lower riparian (RIP_VEG) and higher organic matter content (ORG_MAT) compared to FL sites ($p<0.05$, Table 1). All sites showed similar percentage of water sediment content since sampling was conducted during the first days of the wet season (WAT_CON, Table 1).

As expected, LD sites showed the largest dry period (L_DRY) occurring before the sampling and the shortest rewetting periods (REW) in comparison to the other sites' categories ($p<0.05$, Table 1). However, similar frequency of dry/rewetting cycles was observed among sites, and only MD sites reported significant higher frequencies compared to those of FL sites ($p<0.05$, Table 1).

Influence of hydrological history to streambed microbial functions

The streambed extracellular enzymes and the community respiration were not significantly different between the four hydrological categories (Fig. S1), and their variability was especially high for SD sites in the case of the hydrolytic activities (GLU and XYL) and for the MD and LD sites in the case of the sediment respiration (Fig. S1).

Both variances partitioning (Fig. S2) and averaged models (Table 2) indicated that hydrological variables were relevant for phenol oxidase (PHE) and community respiration (RESP) while the catchment's characteristics explained more of GLU and XYL variability (Fig. S2, Table 2). Specifically, the total duration of the dry phase explained the largest variance in the case of phenol-oxidase activity (PHE, VAR. PAR. T_DRY = 13%, Fig. S2) while the interaction between dry (T_DRY) and duration of last wet period (REW) was relevant for community respiration variation (RESP, VAR. PAR. T_DRY*REW = 13%, Fig. S2). Besides, the organic matter content and the 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. S2).

Table 2. Results of the averaged models derived from Linear Models created for each microbial functional and structural response variable. These models examined the effect of total dry days (T_DRY), the rewetting duration before the sampling (REW), their interaction (T_DRY*REW), and at latest two environmental predictors. Each model's predictors set was selected considering Random Forest analyses results.

GLU	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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	SES	SE	p value	mod.rel.imp.	r^2 (%)
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

Predictors and interaction standardised effect size (SES), standard error (SE) and significance (p value) for each model are shown (significant terms are indicated in bold and by: 0 '***' 0.001 '**'

0.01 '*' 0.05 '.' 0.1). The *na* interaction values indicate not available results automatically excluded from the model because of too low importance. The model relative predictor importance (mod.rel.imp.) and the model's goodness-of-fit (r^2) are also specified for each predictor. Microbial functional and structural variables considered for modelling were: β-glucosidase, GLU; β-xilosidase, XYL; phenol-oxidase, PHE; community respiration, RESP; Shannon diversity index, DIV; richness index, RICH; bacterial density, BAC.

The microbial functions models' plots showed the type of relationship (significant or not) existing between each functional variable and the duration of total dry (T_DRY), and at the same time the interactive effect of the duration of last wet period (REW), in order to define potential additive, synergistic or antagonist effects (Fig. 3). According to the model interaction plots, despite the high variability among the intermittent sites, the hydrolytic GLU and XYL activities slightly decreased when increasing dry length (T_DRY), and this trend was significant in the case of XYL activity (Fig. 3A and B, Table 2). In contrast, phenol-oxidase (PHE) activity experienced a significant increase when dry length increased, showing mean PHE activities doubled in LD compared to those at SD sites (126.63±13.64 and 251.13±55.19 nmolDIQC/gDW*h, in SD and LD sites, respectively), while no interaction with REW was reported (Fig. 3C, Fig. S1 and Table 2). Regarding catchment characteristics, the organic matter content in sediments was positively related to GLU and XYL hydrolytic activities (Table 2) while the riparian vegetation coverage was negatively related to XYL activity (Table 2). Community respiration showed a more complex response to the hydrological conditions, with peaks of increase when longer rewetting occurred in largely intermittent (MD) and extremely dried (LD) sediments (Fig. 3D, Table 2).

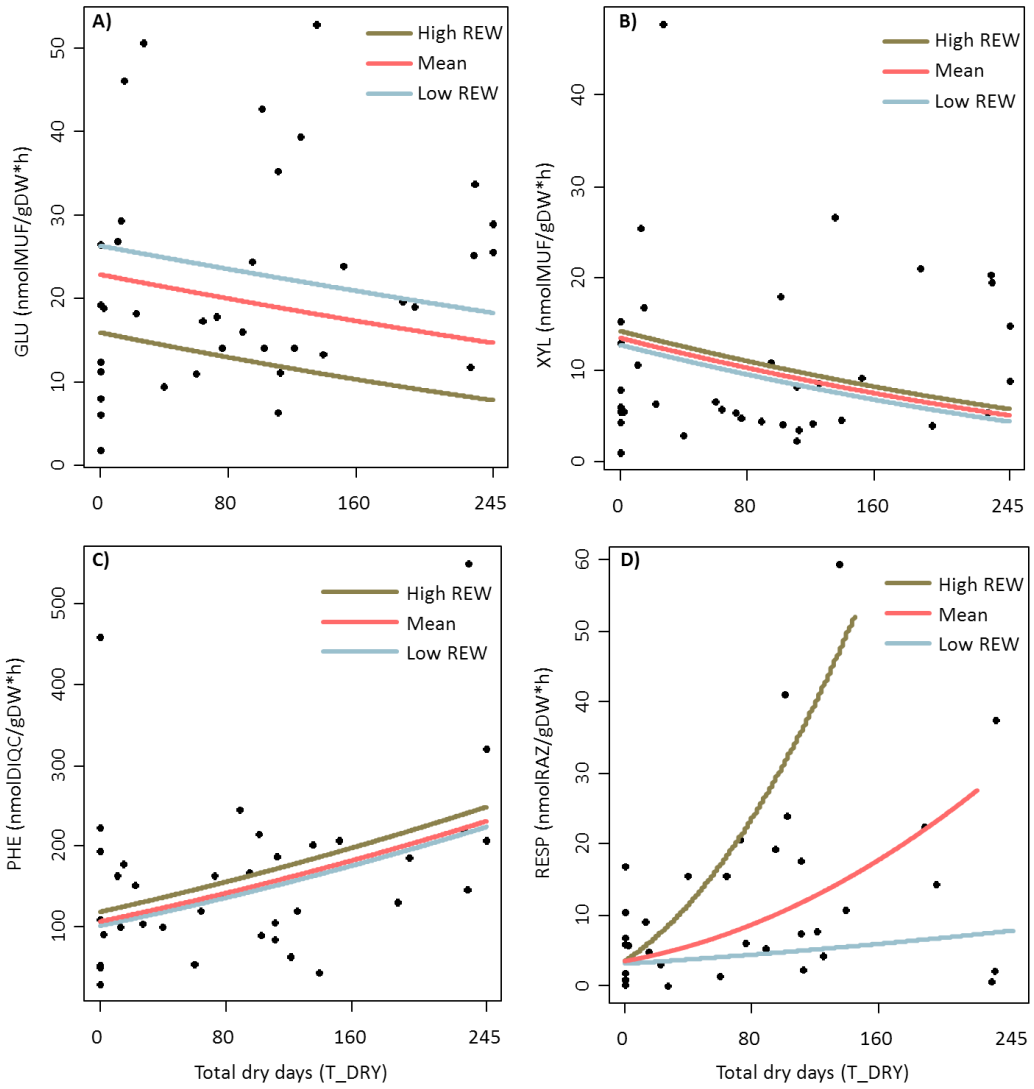


Figure 3. Interaction plots showing the response of the extracellular enzymatic activities (GLU, β-glucosidase; XYL, β-xylosidase; and PHE, phenol oxidase) and the community respiration (RESP) to the hydrology (x-axis, total dry days) and to the duration of the last wet period (REW). The wet duration was fixed at three levels: 5% (low), 50% (mean), 95% (high) percentiles. Each model predictors' selection and interaction were based on the Random Forest analyses (see models' significance at Table 2).

Influence of hydrological history to streambed microbial structure and relationship to function

Remarkably, the sediment microbial community diversity metrics (e.g. Shannon diversity (DIV), richness (RICH), and bacterial density (BAC)), showed stronger relationships with catchment characteristics than to those related to hydrology (Table 2, Fig. S2). The riparian vegetation coverage explained the largest part of the variance in the case of taxonomic diversity (DIV, VAR. PAR. RIP_VEG = 28%, Fig. S2) and taxonomic richness (RICH,

VAR. PAR. RIP_VEG = 33%, Fig. S2) whereas 13% in the case of bacterial density of sediment communities (Table 2 and Fig. S2). A small fraction of the variability was explained by hydrological parameters such as T_DRY, REW and T_DRYxREW, accounting for 5%, 9%, 5% for diversity, richness and bacterial density, respectively (Fig. S2).

In the case of microbial diversity, significant and positive relationships existed between both the diversity variables (DIV, RICH) and the riparian vegetation coverage (RIP_VEG, Fig. 4, Table 2). Averaged linear models showed that abundance of riparian vegetation had an additive interaction with longer duration of dry phase, determining a positive and significant increase of both microbial diversity and richness indices, specifically among the MD sites (Fig. 4A and B; Table 2). With regards to the land-use, the agriculture land coverage of the watershed explained the largest part of the bacterial density variance (BAC, VAR. PAR. AGRI = 33%). The average models reported that agricultural land use was positively and significantly related to the bacterial density, but negatively related with the community diversity (Table 2).

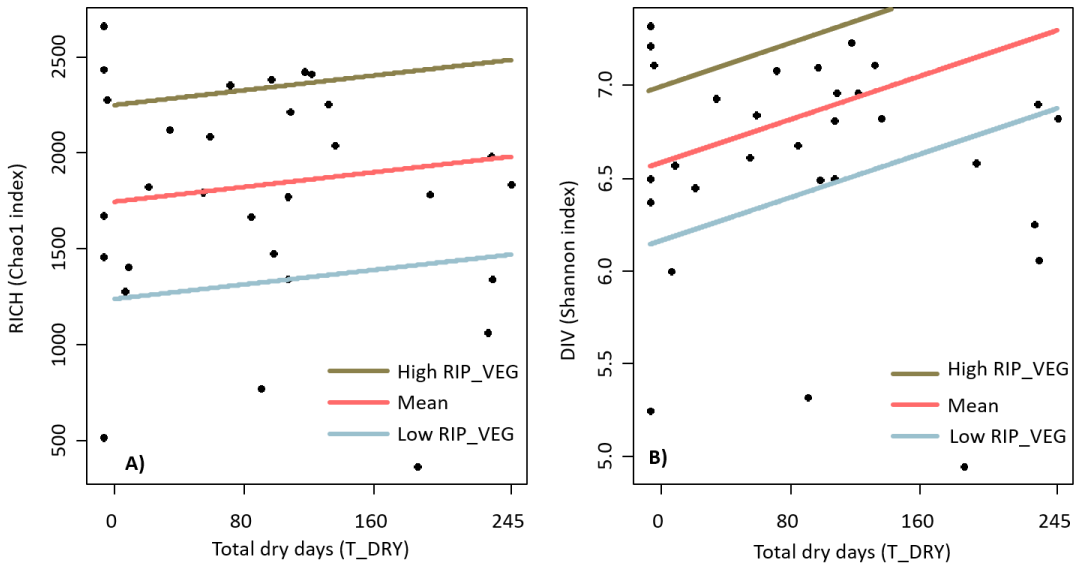


Figure 4. Interaction plots showing the response of the microbial taxonomic diversity (DIV, Shannon-Wiener index) and richness (RICH, Chao1 index) to the hydrology (x-axis, total dry days) and to the duration of the last wet period (REW). The wet duration was fixed at three levels: 5% (low), 50% (mean), 95% (high) percentiles. Each model predictors' selection and interaction were based on the Random Forest analyses (see models' significance at Table 2).

The bacterial community composition in sediments changed in response to the duration of the dry phase, and significant differences were found between communities at the flowing sites (FL) and those at the intermittent sites (SD, MD, and LD) (PERMANOVA, Fig. 5). In terms of taxonomic community composition, the phylum Proteobacteria dominated the streambed

communities from all sites, though its relative abundance decreased at the intermittent sites (SD, MD, LD Fig. S3). Specifically, a significant reduction in Delta-proteobacteria and Epsilon-proteobacteria classes was reported from FL to LD sites (ANOVA $p<0.05$; Fig. S3, Fig. 5). Similarly, the relative abundance of phylum Planctomycetes decreased along the dry phase gradient, and showed significant decrease in Planctomycetia class relative abundance in MD and LD sites compared to that of FL sites (ANOVA $p<0.05$; Fig. S3, Fig. 5).

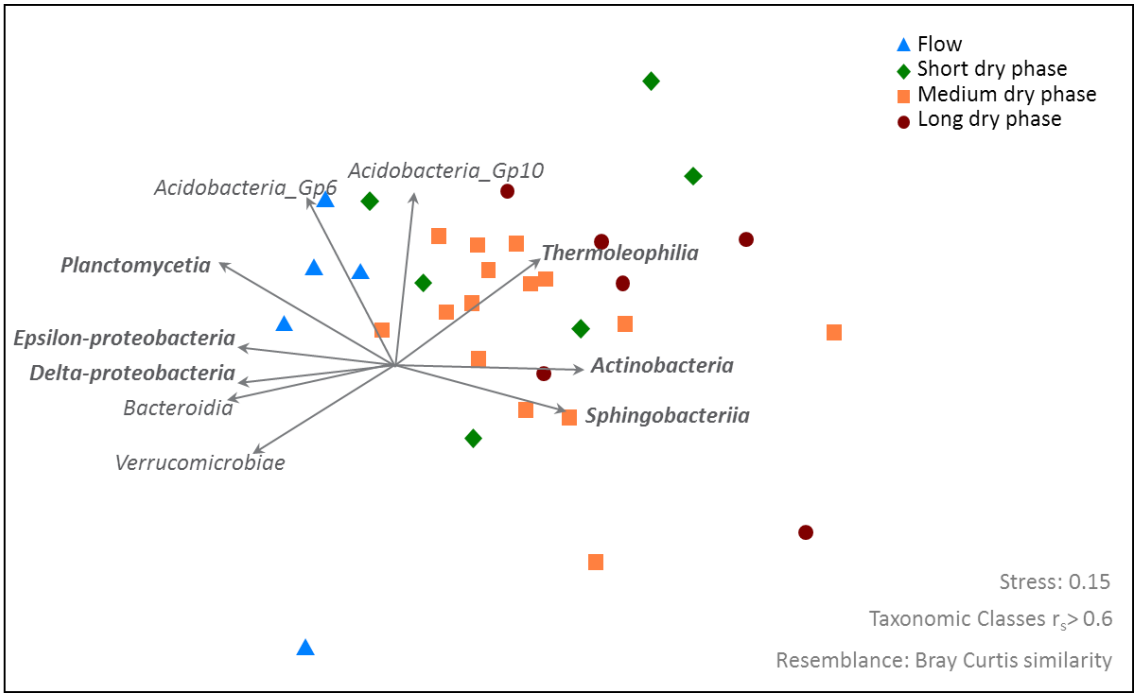


Figure 5. NMDS ordination's plot representing the bacterial community composition (OTUs abundance) in the study sites (coloured by hydrological category, legend). The arrows indicate the correlation with taxonomic classes (Spearman coefficient $r_s>0.6$). Those in bold resulted significantly different in the relative abundances between FL and MD/LD sites (ANOVA $p < 0.05$). The PERMANOVA test of the NMDS ordination analysis and subsequent pairwise comparison indicated significant differences between hydrological categories ($p=0.003$) where the flow site communities were significantly different to all other three intermittent bacterial communities ($p=0.051$, $p=0.001$, $p=0.001$ for FL-SD, FL-MD, and FL-LD comparisons, respectively). There were no significant differences between the three intermittent categories ($p>0.1$ for all comparisons).

In contrast, the relative abundance of Actinobacteria and Bacteroidetes doubled in sites with largest dry phase (Fig. S3). More specifically, strong correlations of Actinobacteria ($r_s=0.76$), Thermoleophilia ($r_s=0.56$), and Sphingobacteria ($r_s=0.65$) classes and the MD and LD communities were found (Fig. 5), together with significant increase in Actinobacteria relative abundance in MD and LD sites (ANOVA, $p<0.05$; Fig. S3, Fig. 5), and significant increase in Sphingobacteria relative abundance in LD sites, compared to those of FL sites (ANOVA, $p<0.05$;

Fig. S3, Fig. 5). The global correlation between the community composition matrix and the functions matrix was not significant ($p = 0.182$, $r_s=0.10$), revealing that changes in community's taxonomy did not explain shifts in their functions (Supplementary Information S3).

DISCUSSION

Under persistent dry conditions, streambed sediments are totally exposed to the atmosphere and may acquire characteristics similar to those of adjacent soils (Arce et al., 2019; Morandi et al., 2014; Steward et al., 2012). The expansion of the dry phase duration and the alteration of dry-wet cycle frequency in river ecosystems often determine the degree of similarity between streambed and terrestrial habitats (Harms and Grimm, 2012; Mori et al., 2017). Generally, prompt reductions in microbial activities, changes in diversity and shifts of the communities' composition are expected as the drying period enlarges (Amalfitano and Fazi, 2008; Marxsen et al., 2010; Rees et al., 2006) although also co-occurring environmental factors are often referred as important modulators for microbial responses to drought (Drenovsky et al., 2010; Ruiz-González et al., 2018). In the present study, the functions, diversity and composition of streambed microbial communities displayed different relationships with the hydrological history, but also with the catchment characteristics. Overall, microbial communities' composition was responsive to the water stress gradient whereas microbial communities' diversity was almost maintained. A progressive microbial functional shift was observed along the dryness gradient and community assemblages showed a step change from flowing to intermittent sites. Notably, the microbial community diversity was mostly related to the environmental features of the catchment (riparian vegetation coverage and agricultural land use) rather than to hydrological influences.

Drying gradient affecting streambed microbial function

Similar to previous studies, we found that the presence of specific extracellular enzyme activities was influenced by the extended duration of the dry phase (Zoppini et al. 2014, Freixa et al. 2016, Gionchetta et al. 2019). At increasing dryness conditions phenol oxidase activity was enhanced in detriment of β-xylosidase, indicating an increased capability to degrade lignin compounds but reduced capability to degrade simple polysaccharides from plants (hemicellulose derived). This shift of enzyme capabilities suggests a gradual modification of the quality of organic matter available in streambed sediments when getting dry (Fig. 6). According to previous studies, the reduced flow connectivity, the accumulation of old plant litter material 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 reduced inputs of freshly available organic compounds as those produced by algae in wet conditions (Acuña et al., 2007; Freixa et al., 2016; Romaní et al., 2013).

Surprisingly, β-xylosidase (XYL) activity was negatively related with riparian vegetation, although this enzyme is usually involved in the degradation of plant origin hemicellulose (Romani et al., 2006, 2013). The large variability observed in the enzymes activity among the intermittent sites may indicate a high plasticity of the microbial community to changing organic matter sources use. The microbial enzyme activity may be a trade-off between available resources and their quality. For instance, when simple polysaccharides from algal activity are available together with allochthonous plant material from riparian vegetation, GLU would be the favoured enzyme produced in front of XYL since end product is glucose (more energetically relevant than xylose). The mismatch between XYL and riparian vegetation may be further due to the high XYL activity among the intermittent sites (usually with lower riparian cover) being favoured by the reduced availability of readily degradable algal released compounds, due to the intermittency effect on algal metabolism (Fig. 6). Overall, these results suggested that the coupled quantity and quality of organic matter influenced the microbial capacities to use the organic materials and show an adaptation to the sources available due to changes in intermittency and co-occurring factors such as riparian vegetation coverage (Fig. 6). Clearly, long dry-phase sites (LD) were characterized by the use of more terrestrial origin materials (especially shown by the increase in phenol-oxidase activity, PHE) and also showed much lower riparian vegetation (Table 2, Fig. 6).

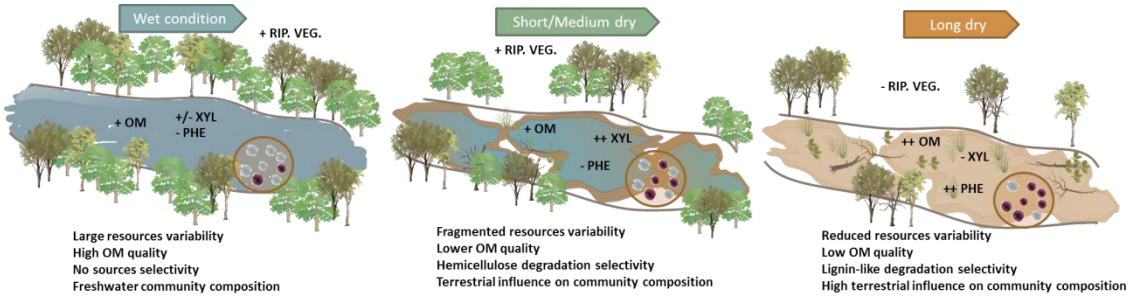


Figure 6. Conceptual diagram summarizing the main results obtained for the intermittent streams studied. The quantity and quality of the organic matter (OM) and the abundance of riparian vegetation (RIP.VEG.) apparently drove the selection of specific degradation enzyme utilization (such as the hydrolytic β-xylosidase, XYL, or the recalcitrant activity of phenol-oxidase, PHE). Shifts in bacterial community composition through drought-resistant taxa increase under prolonged desiccation are illustrated in the circles (violet and grey colours refer to gram positive and negative bacteria, respectively).

Besides dry-length effect, the presence of wet episodes may further affect microbial activity by, in contrast, enhancing it. This was observed in the case of respiration activity which was especially enhanced when long rewetting occurred in hardly dried sites. Also, the two hydrolytic enzyme activities experienced high variability among the sites with short and intermediate length of dry period (SD and MD, Fig. S1). The large variability of these activities suggested that greater intermittency (i.e. alternation of dry and wet episodes) may determine larger microbial activities fluctuations, which in turn could have masked the expected decrease tendency of β-glucosidase activity along drying gradient. Indeed, wet events can break the constant increase of aridity and make available simpler and more labile compounds, mobilizing them into the sediment (Birch and Griffiths, 1961; Fierer and Schimel, 2003; Rees et al., 2006). This process, known as Birch effect, is usually observed in arid soils consisted in pulses of nutrients and labile carbon, caused by the release of intracellular components from microbial cells osmotically stressed by the rapid rise in water potential (Birch, 1958; Placella et al., 2012). Here, the consequences of the last wet episode may have reactivated a large number of dormant microbes (Timoner et al., 2012) or of still-active oxidative enzymes presented in damaged or dead cells (Burns et al., 2013; Meisner et al., 2015). Overall, our results suggested similar to soil microbial community performance in terms of respiration activity although no associated increase of bacterial biomass was observed, suggesting that the microbial growth may re-start after a lag-time (Meisner et al., 2015).

Drying gradient affecting streambed community structure and composition

Microbial community composition reflected clear differences between flowing and intermittent sites, which suggests a relevant effect of having no-flowing episodes irrespectively of the degree of intermittency. Microbes are recognized nimble entities, being able of rapid adaptation to changing environmental conditions through changes in diversity, composition and functions (Allison and Martiny, 2008; Wallenstein and Hall, 2012). Consequently, the stress provoked by a single dry period may determine changes on intermittent microbial communities through cells rapid osmotic adaptation in order to cope with intermittent periods' alternation (Attermeyer et al., 2013; Meisner et al., 2018; Shade et al., 2012). As expected, the increased dryness tended to modify the microbial communities with respect to those of the flowing sites, involving rises in relative abundance of drought-tolerant taxa in detriment of drought-sensitive taxa. This microbial community transition was characterized by a decrease of Gram-negative bacteria against an increase in the proportion of Gram-positive bacteria, which are more resistant and adapted to live in soil systems submitted to extreme drought (Acosta-Martínez et al., 2014; de Vries and Shade, 2013; Naylor, 2017).

Furthermore, the microbial composition changes were driven by only one or few members of a phylum within the microbial community, as similarly observed by Barnard et al. (2013) in soil systems. Specifically, here in intermittent streambeds, assemblages' shifts included significant decrease in the Gram-negative classes of Epsilon-proteobacteria and Delta-proteobacteria (Proteobacteria phylum) and Planctomycetia (Planctomycetes phylum). Conversely, we found significant increases in the Gram-positive Actinobacteria and Thermoleophilia classes (phylum Actinobacteria) as observed in soils under extreme desiccation (Bouskill et al., 2013). The spread of Sphingobacteria class (Bacteroidetes phylum) among the intermittent driest communities might be due to the effect of rewetting (last rewetting event before sampling). Sharp increases of Sphingobacteria class relative abundance has been usually associated to abrupt wet events occurring in arid and desert soil communities (Aslam et al., 2016; Meisner et al., 2018). In contrast to community composition, richness and diversity was not significantly modified by hydrological history, suggesting that there was taxa-dominance alternation or substitutions without significant effect of increased dryness on the overall diversity.

Relevance of the environmental catchment characteristics

Riparian vegetation and land use (agricultural coverage) were significant drivers for the streambed microbial community richness and diversity. While riparian vegetation positively influenced microbial community diversity, the agricultural coverage negatively affected it but enhanced bacterial density. The riparian vegetation may help on the conservation of the stream biodiversity and ecosystem functioning (Elosegi et al., 2010). The larger bacterial diversity and richness in association with the greater riparian vegetation coverage might be related to the increase of available microhabitats for microbes (e.g. through leaf fall, pools persistence, terrestrial-plants colonization) (Arce et al., 2019; Romaní et al., 2017). On the other hand, although no direct effect of hydrological history has been found for community diversity, in our study the reduced riparian vegetation co-occurred with drying length. This suggests that desiccation may also play a role in the decreasing diversity and richness when decreasing riparian vegetation cover.

Regarding land use, the agricultural coverage was the major force reducing the streambed bacterial diversity but at the same time increasing bacterial density. Even though most of the studied sites presented similar catchment characteristics, some of them presented large coverage of agricultural fields surrounding the study sites. These microbial communities' responses might be consequences of the enhanced nutrient inputs from agricultural practices surrounding the studied sites, which could induce bacterial growth and the spread of some dominant species and thus reducing diversity as expected in eutrophic conditions or in nutrient-contaminated systems (Li et al., 2018; Zeglin, 2015).

Furthermore, the negative effect of agricultural cover on bacterial diversity may be further enhanced by drought length (negative interaction T-DRYxAGRI, Table 2), as also suggested in previous studies with leaf litter decomposition and soils (Duarte et al., 2017; Meisner et al., 2018).

Functional vs structural streambed responses and concluding remarks

Embracing both microbial function and community composition streambed responses, these findings highlight a significant effect of hydrological history although function and diversity responses were not occurring in parallel (absence of correlation in Mantel test). Interestingly, functions, in terms of organic matter use capabilities (i.e. extracellular enzymes) showed a gradient response, increasing the use of lignin compounds but decreasing the use of simple polysaccharides in streambeds suffering longer dry periods as well as high variability within intermittent sites. In contrast, streambed community composition showed a step change when flowing waters became intermittent irrespective on the drying intensity, while richness and diversity were strongly modulated by the catchment characteristics. Contradictory results are also presented in literature, where some studies reported coupled structural (diversity and composition) and functional microbial responses to water stress, while others showed activity responses to desiccation decoupled from changes in microbial communities' structure and/or composition (Febria et al., 2015; Frossard et al., 2012; Pohlon et al., 2013a; Zoppini et al., 2016).

The intrinsic high variability of a field assessment could explain the unexpected weak correlation observed between microbial community composition and functions, bearing in mind that the activities and diversity measured here were not exhaustive for explaining the overall system functions and structure potential correlations. Overall, the maintenance of the microbial diversity under extreme dry streambed condition, potentially ensures reduced loss in the system functioning. Nevertheless, more studies are needed to confirm whether longer dry periods combined with reduced riparian vegetation and increase in the agricultural land use would end up endangering the microbial mediated processes linked to the carbon cycle.

5. GENERAL DISCUSSION

Nothing in biology makes sense except in the light of evolution.

Theodosius Dobzhansky

This section provides a summary of the main findings, a broader discussion with a global perspective that integrates all the results from the studies presented, their implications and other relevant aspects.

Water scarcity, loss of freshwater ecosystems, and drought are among the most pressing environmental issues of the current century (Collins et al., 2009). Freshwater ecosystems, already damaged by the overuse of water by growing populations, stand to be further affected by climate change, with widespread shifts in rainfall patterns that are likely to intensify droughts in some regions.

Across Europe, the frequency and intensity of droughts has already increased dramatically over the past thirty years (Collins et al., 2009). Mediterranean intermittent rivers are among the most impacted by climate change and human water needs, and despite their natural flow-intermittent behaviour, the reality of the water scarcity is endangering their status moving closer to a terrestrial system (Datry et al., 2017a). Worldwide many researchers investigate the consequences of drought and the functioning of the intermittent freshwater ecosystems, to better evaluate whether the transition to a terrestrial-like system is an occurring reality.

Intermittent stream sediment ecotone contains unique microbial diversity including assemblages more or less adapted to the hydrological fluctuations which are relevantly involved in several biogeochemical in-stream processes (Barthès et al., 2015; Logue et al., 2016). Unlike streambeds of permanent flowing watercourses, streambeds from intermittent watercourses are transitional habitats acquiring similar features to nearby soils passing from wet to extremely dry conditions (Arce et al., 2019; Elosegi et al., 2010; Morandi et al., 2014; Thorp et al., 2006). The degree of similarity with soils is highly linked to the duration of the dry phase and the rewetting episodes frequency (Harms and Grimm, 2012; Mori et al., 2017) as well as to a corollary of environmental factors (e.g. solar radiation, sediment water retention capacity, shadow cover) that can contribute to preserve or not the aquatic features of the streambed. Nowadays, drought and rewetting episodes as well as punctual storms interrupting prolonged desiccation periods are sharpened and this could contribute to endanger the microbial biodiversity and functioning (Findlay, 2010). Contrasting results are recently published about the capacity of the microbial communities inhabiting the intermittent streambed to conserve their functions and structure to the wet-dry-rewet alternations (Baldwin and Mitchell, 2002; Barthès et al., 2015; Febria et al., 2015; Romaní et al., 2014). In terms of intermittent stream ecosystem conservation, changes in microbial functions related to the nutrients cycling can be considered more alarming than potential coupled or uncoupled modification in the community biodiversity. The main objective of this thesis was to understand how microbial communities (prokaryotes and eukaryotes) inhabiting intermittent streambeds respond to desiccation, in terms of structure, functions and composition.

Overall the results from this thesis showed a functional shift in the organic matter utilization, a specific structural-strategy response (i.e. extracellular polymeric substances, EPS, production)

to cope with extremely dry conditions, and changes in the relative abundance of certain microbial taxa reflecting the community composition modification influenced by the different streambed hydrological states. Overall, a clear shift in the organic matter degradation capacity was observed in superficial streambed experimentally and naturally submitted to long dry phase (Chapter 1 and Chapter 4). Additionally, differences in microbial organic matter degradation and communities' composition changes were observed among the distinct type of micro-habitats (surface and hyporheic sediment and buried leaves), and these changes were mainly related to the sediment humidity and oxygen availability of the habitats (Chapter 1 and Chapter 2). Furthermore, variation in the relative abundance of drought-sensitive, drought-resistant taxa was also observed in the superficial sediment across the long- dry phase (Chapter 2 and Chapter 4). On the other hand, the overall microbial diversity, measured through indices, appeared more sensitive to catchment characteristics and land use (e.g. reduced riparian vegetation and agricultural impact) than to desiccation (Chapter 4). Regarding the methodological aspects, the functional (as Extracellular Enzyme Activities and Community Level Physiological Profiles) and molecular (as microbial DNA characterization) tools applied in this thesis confirmed their usefulness as ecological descriptors of the hydrological impacts influencing the streambed microbial communities (Chapter 3).

In this general discussion it is aimed at integrating the specific results from the different chapters by comparing the results obtained by the two main experimental approaches: the specific laboratory experiment in microcosms and the field multiple-site multiple-catchment approach. The experimental approach allows us to fix/control experimental conditions and isolate the specific effect of dryness, while the results from the field study embrace natural hydrological alterations. This comparison will help on our better understanding about expected responses of streambed microbes to drought as well as better identifying key environmental drivers that may modulate their response.

This comparison has brought us to the following **discussion nodes**:

5.1. Altered hydrology and intermittency cycles push streambed microbial communities to select their activities and alter their taxa-dominance, determining:

5.1.1. Functional shifts

5.1.2. Diversity and taxonomic composition variability

5.2. The relevance of the different approaches and methods for microbial community composition and function: Are we telling the truth?

5.3. Resistance to climate-anthropogenic hydrological changes; driving forces and responses at different scales:

5.3.1. Microbial-scale: EPS production

5.1. *Altered hydrology and intermittency cycles push streambed microbial communities to select their activities and alter their taxa-dominance*

The study of the consequences of the transitions from wet to dry and from dry to wet on the stream microbiota is the fundamental core of most research in intermittent stream ecology. In the context of shifting environmental conditions, the observation of potential changes occurring in the streambed microbiota is of paramount relevance because of their functions in mediating ecosystem processes (most related to water quality, nutrient processing, gases emission), and because of the importance of preserving the microbial genetic diversity supporting those functions (Febria et al., 2012; Zeglin, 2015). For instance, many studies identified the hydrological phases' alternation (from wet to dry) driving the change in the quality and quantity of organic matter available in the sediment and the water and nutrients availability, being major forces determining shifts in the potential extracellular enzyme activities expressed by microbial communities (Freixa et al., 2016; Pohlon et al., 2013a; Timoner et al., 2014a; Ylla et al., 2009). Other studies reported modifications of microbial diversity uncoupled to functional shifts or vice-versa, reflecting cases of functional plasticity (maintain community composition but modify functions) or functional redundancy (alter composition but not the community functions), or simply cases of great functional resilience, once the flow condition was recovered (Allison and Martiny, 2008; de Vries and Shade, 2013; Shade et al., 2012; Simon et al., 2016; Zeglin, 2015). Many researches supported the theory of the phylogenetic conservation of microbial functions, already used to cope with environmental changes, which could derive to functional redundancy without variation of microbial diversity (Battin et al., 2016; Griffiths and Philippot, 2013). Thus, contradictory results are present in literature, where some researchers reported coupled structural and functional microbial responses to water stress, while others showed activity responses to desiccation decoupled from changes in microbial communities' structure and/or composition (Febria et al., 2015; Frossard et al., 2012; Pohlon et al., 2013a; Zoppini et al., 2016). It is then evident that there is a great uncertainty in the extent to which intermittent streambed microbiota is sensitive or resistant to environmental hydrological perturbations (Allison and Martiny, 2008; Shade et al., 2012; Zeglin, 2015). Here, results from the microbial functions and structure (diversity and composition) obtained from the long-term experiment and the multi-site field approach are related and compared.

5.1.1 *Functional shifts*

Disparities in organic matter quality and quantity between the different hydrological phases may translate into differences in organic matter decomposition capabilities (Ylla et al., 2010). In general, a drying streambed may accumulate greater amount of organic matter (e.g. from animal or vegetal debris or augmented accumulation of leaves fall) compared with other hydrological states and with the adjacent soils, especially in arid, semiarid and Mediterranean regions where the vegetation is limited to the riparian zones (Arce et al., 2019; Fossati et al., 1999; Steward et al., 2012). Even under dry conditions extracellular enzyme activities (EEAs) have been measured and related to the microbial organic matter decomposition in stream sediments (Marxsen et al., 2010; Zoppini et al., 2014). Previous studies also linked the type of enzyme activities activation to the distinct hydrological phases (droughts vs flooding) in intermittent rivers (Freixa et al., 2016; Romaní et al., 2013; Ylla et al., 2010). The results from Chapters 1 and 4 of this thesis seemed to separate enzymatic activities between the wet streambeds from extremely dry streambeds (dry phase larger than 5 months), and this was observed in both the laboratory and field approaches (Fig. 1).

In this thesis, higher utilization of simple polysaccharides was observed in the wet period which specifically corresponded to the initial wet condition of the experiment (Initial exp. In the Figure 1) and the Flow sites sampled (Fig. 1A and 1B), in contrast to the long-dry experiment. Similarly to previous studies, cellobiose degradation activity (β-Glucosidase, GLU) showed high variability and its activity has been maintained independently from the duration of the dry phase, especially in the case of the field observations (Fig. 1A) (Freixa et al., 2016; Timoner et al., 2012). Contrariwise, the laboratory experiment results revealed a decrease in GLU activity under prolonged dry phase, but greater GLU activity variability when punctual storms occur, similarly to that observed among short (SD) and medium (MD) intermittent sites (Fig. 1A). These results underlined the importance of the wet events moistening the dry sediment which limited the decrease of the activity probably because of the release of more labile compounds occurring during the wet episodes (Evans and Wallenstein, 2012; Fierer et al., 2003b; Shumilova et al., 2019). Furthermore, in the pool-like conditions significant peaks of GLU activity (Fig. 1A) have been associated to the algal colonization, typically occurring in these microhabitats, therefore considered as a microbial refuge under harsh conditions (Casas-Ruiz et al., 2016; Larned et al., 2010).

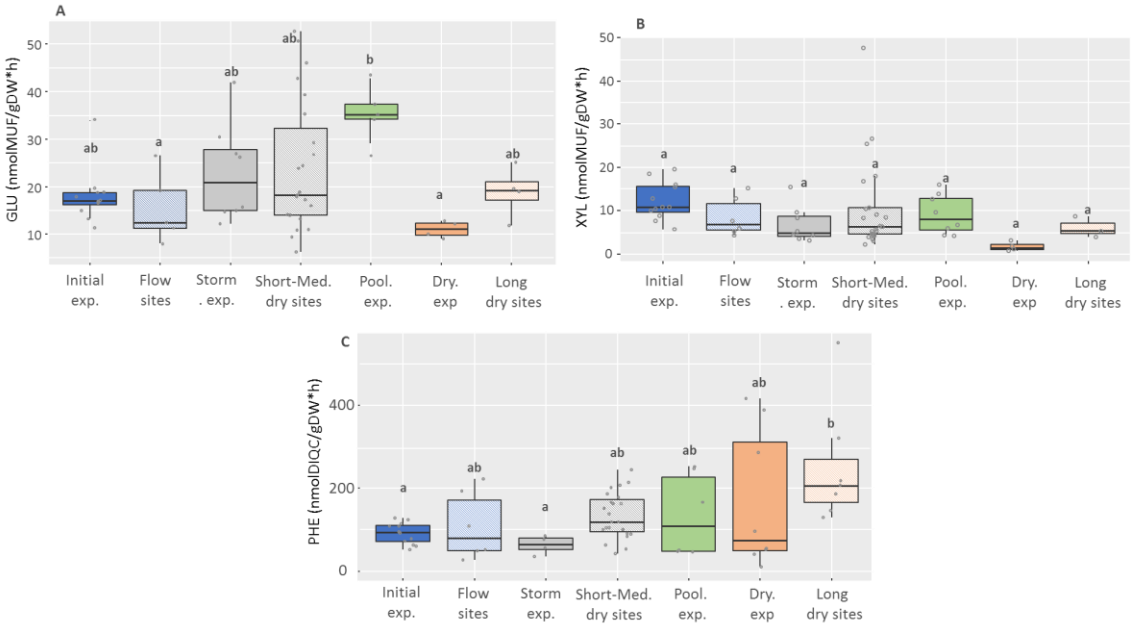


Figure 1. Box plots of extracellular enzyme activities: A) β-Glucosidase, GLU, B) β-Xylosidase, XYL and C) Phenol-oxidase, PHE. Data were collected from different Chapters (1 and 4) corresponding to experimental (exp.) and field (stream sites) studies. Initial (Time 0), Storm (Time 150 of Dry-Storm treatment), Pool (Time 90 and 150 of Control treatment) and Dry (time 150 of Dry treatment); data correspond to the experiment results from the superficial sediment (Chapter 1). Flow sites, Short and Medium dry phase sites and Long dry phase sites data correspond to the surface sediment streambeds studied in the field approach (Chapter 4). The texture indicates the experimental results whereas the letters indicate significant differences between the categories tested with one-way ANOVA.

Under prolonged desiccation periods the results from β-xylosidase (XYL) and phenol-oxidase (PHE) activities performed opposite patterns (e.g. reduction of XYL and increase of PHE), reported from both experimental and field studies (Fig. 1B, 1C), but significant in the case of PHE. In the case of XYL, the slight reduction observed under extreme dry condition (Fig. 1B), was related to the potential change of the quality of the organic matter stored in the surface sediment during the desiccation phase (Romaní et al., 2012; Ylla et al., 2010). This pattern was especially clear for the laboratory experiment (Drought treatment) whereas in the field work this tendency (LD sites) was weaker, probably because sampling was conducted at the beginning of the wet season in all the studied sites. Contrarily, PHE activity resulted in a significantly increase when increasing dryness conditions in the streambed (Fig. 1C). In the dried streambeds the greater accumulation of allochthonous debris mostly composed by recalcitrant materials would enhance degradation of lignin-like materials (Burns et al., 2013; Romaní et al., 2006a; Sinsabaugh, 2010).

Importantly, the XYL and PHE enzyme activities were the most responsive enzymes to the desiccation experiment and to some extent also to the natural intermittency gradient. Bearing

in mind the complexity and the multiple factors that could influence the microbial functional response in field approaches, these enzymes could be considered as useful microbial functional markers for the detection of extreme drought conditions, behind which the resilience of the microbial-mediated ecosystem functions could be compromised. In particular, the greater utilization of PHE among the intermittent streambeds, submitted to long-term drought, could be used as a reference for potential begin of transition from freshwater to terrestrial systems.

5.1.2 Diversity and taxonomic composition variability

The hydrological fluctuations from basal to extremely dry condition fragment the intermittent river path creating microhabitats which could be recognized as microbial refuges (Romaní et al., 2017). The stream path fragmentation and the variation of the quality and quantity of organic matter have been proved to change the streambed microbial diversity and composition (Freixa et al., 2016; Marxsen et al., 2010). Streambed community composition changes due to changing hydrology to greater intermittency may be also linked to functional changes (as those described above), but, as previously mentioned, contradictory results rose when investigating whether microbial functional shifts were associated to diversity and composition changes. The streambed bacterial diversity (measured as diversity indices) analysed in this thesis tended to decrease when increasing the duration of the dry phase (Figs. 2A and 2B). In spite of the large variability observed among the results obtained, significant diversity reductions have been reported under long dry period, especially from the laboratory results (Figs. 2A and 2B).

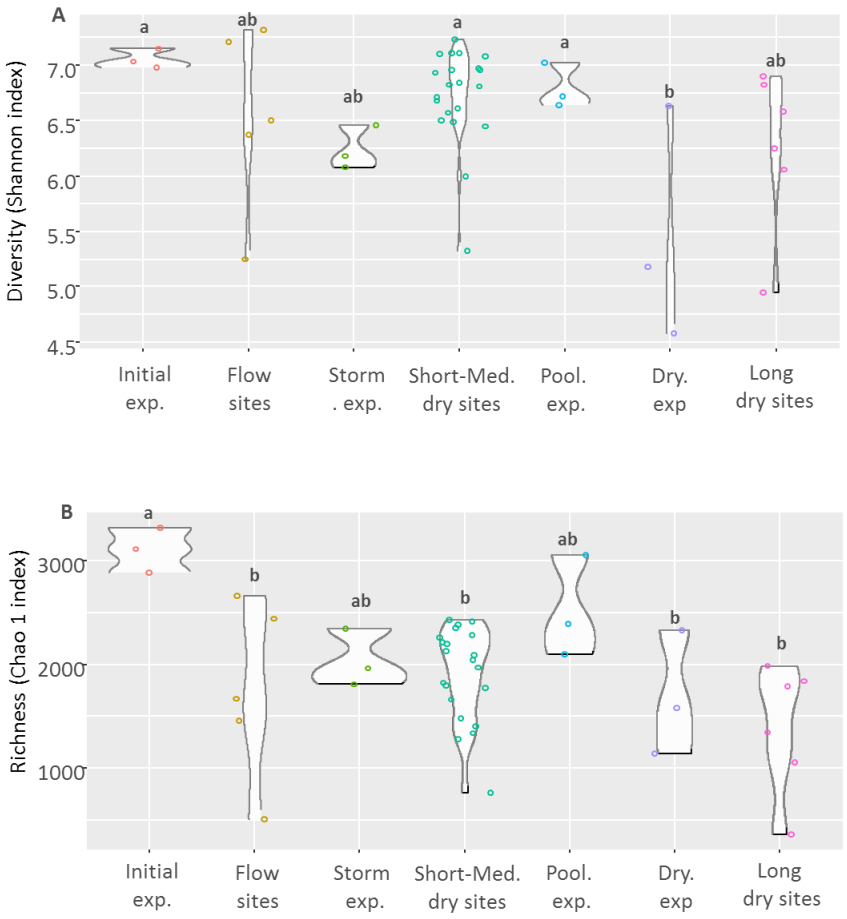
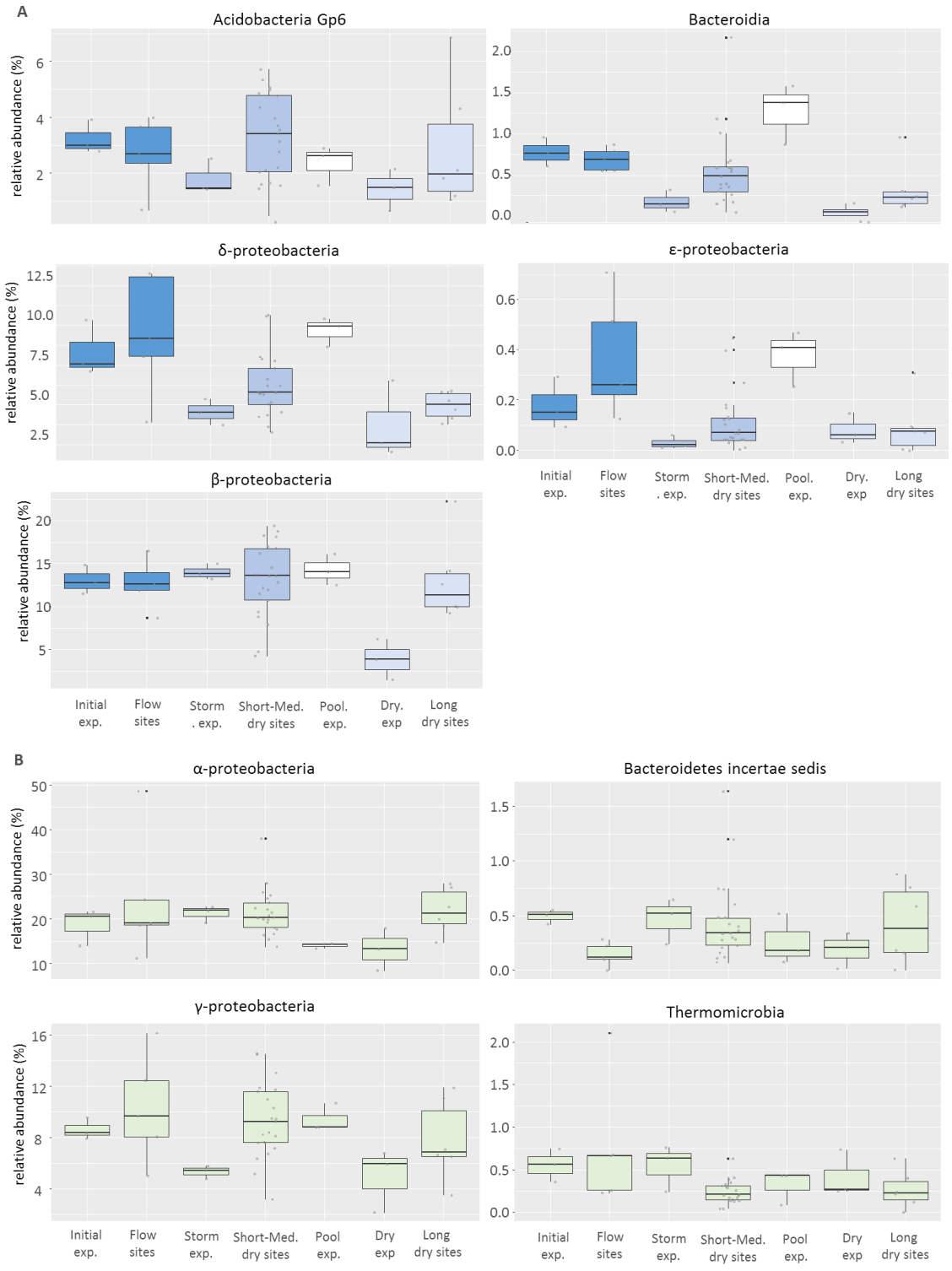


Figure 2. Box plots of bacterial diversity indices: A) Shannon-Wiener diversity index B) Richness Chao1 diversity index. Data were collected from different Chapters (1 and 4) corresponding to experimental (exp.) and field (stream sites) studies. Initial (Time 0), Storm (Time 150 of Dry-Storm treatment), Pool (Time 90 and 150 of Control treatment) and Dry (time 150 of Dry treatment) data correspond to the experimental results from the surface sediment (Chapter 1). Flow sites, Short and Medium dry phase sites and Long dry phase sites data correspond to the streambeds studied in field (Chapter 4). The letters indicate significant differences between the categories tested with one-way ANOVA.

In terms of bacterial taxonomic composition, changes in the most abundant classes colonizing the streambed were observed during the distinct hydrological conditions in both the laboratory and field studies (Fig. 3). Specifically, results reported the transition of the classes' abundance from those that clearly reduced their abundances when increasing drought duration, (e.g. β- and δ-Proteobacteria and Bacteroiidia, Fig. 3A) which could be defined as "drought sensitive", to those classes that increase their abundances and were mainly found in sites submitted to long-drought (e.g. Actinobacteria, Bacilli and Thermoleophilia Fig. 3C) which could be defined as "drought-adapted". Other classes were not significantly modifying their

abundances due to drought (e.g. Bacteroidetes and α- and γ-Proteobacteria, Fig. 3B) and could be defined as “drought resistant”. These findings, observable among both laboratory and field experiments, supported that drying stress in stream sediment is a relevant driver for changes in the microbial communities’ structure. As previously observed from different studies on soil and streambed microbial communities, hydrological changes could influence the taxa selection (e.g. abundance of specific classes) able to cope with osmotic stress (Fierer and Schimel, 2003; Romaní et al., 2013; Schimel, J., 2007; Zoppini et al., 2014). The “drought-adapted” classes observed in our studies have been already identified as able to cope to desiccation periods in previous research mainly focused on soil microbial composition changes under drought conditions (Barnard et al., 2014, 2013; Manzoni et al., 2012; Meisner et al., 2018; Naylor and Coleman-Derr, 2018; Schimel, J., 2007). Furthermore, recent research reported similar adaptation of bacteria and fungi inhabiting soils and dry streambed submitted to frequent and intense dry conditions, such as the thicker Gram positive bacterial and fungal cell walls in order to better cope with the osmotic stress (Jones and Lennon, 2010; Manzoni et al., 2012; Yuste et al., 2011; Zeglin, 2015).


A
Acidobacteria Gp6
Bacteroidia
δ-proteobacteria
ε-proteobacteria
β-proteobacteria
relative abundance (%)
Initial exp.
Flow sites
Storm . exp.
Short-Med. dry sites
Pool. exp.
Dry. exp
Long dry sites
B
α-proteobacteria
Bacteroidetes incertae sedis
γ-proteobacteria
Thermomicrobia
Storm exp.
Pool exp.
Dry exp

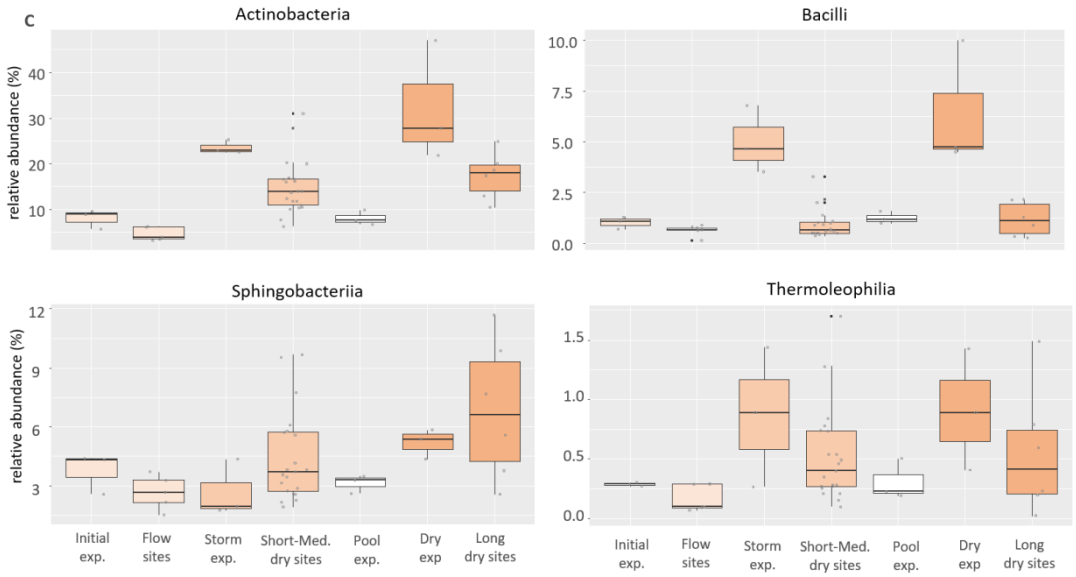


Figure 3. Box plots of the 13 most abundant taxonomic classes found in both the experiment and field studies: A) Drought-sensitive (decreasing relative abundance when increasing drought length), B) Drought-adapted (increasing relative abundance when increasing drought length) and C) Drought-resistant (not modified in the relative abundance when increasing drought length). Data were collected from different Chapters (2 and 4) corresponding to experimental (exp.) and field (stream sites) studies. Initial (Time 0), Storm (Time 150 of Dry-Storm treatment), Pool (Time 90 and 150 of Control treatment) and Dry (time 150 of Dry treatment) data correspond to the experimental results from the surface sediment (Chapter 2). Flow sites, Short and Medium dry phase sites and Long dry phase sites data correspond to the streambeds studied in the field (Chapter 4).

All of these evidences strengthened the similarity between the dry streambed systems studied and a (dry)-soil environment, suggesting that prolonged and unusual dry periods could boost the terrestrial transition of the aquatic intermittent ecosystem.

5.2 *The relevance of the different approaches and methods for microbial community composition and function: Are we telling the truth?*

The combination of the long-term laboratory experiment (Chapters 1 and 2) and the natural observations (Chapter 4) allowed envisaging the advantages and disadvantages of both the approaches. Laboratory experiments generally offer a certain degree of experimental control. For instance, the advantages of using columns of stream sediment are the replicability and the ability to control and simulate the conditions of streambed submitted to long desiccation. The simplification of the field conditions derives to easier understanding of the ecological patterns and processes (Jessup et al., 2004). On the other hand, the over simplified as well as the over reduction of spatial and temporal scales constitute the main disadvantages

of the laboratory approximations. Therefore, extrapolation and interpretation of experimental results to natural aquatic ecosystems must be done with caution. Contrarily, field observations offer the reproduction of the total natural complexity but for this reason is quite difficult to underline and obtain clear patterns and conclusions. In this context, a proper data analysis as multiple factors modelling (Chapter 4) or comparative approach (Chapter 3) would be very useful when dealing with large environmental variability.

Here, the integrated comparison between these approaches resulted in a more accurate description of the complex microbial dynamics existing in intermittently dried streambeds (Fig. 4). In addition, the methods comparisons of the functional and molecular tools used and their relation to the microbial responses considered, was assessed in order to suggest the most adequate metrics among those applied (Chapter 3). In this context, the extracellular enzyme activities (EEAs) were based on short incubation of the natural sample with an artificial fluorescent substrate (e.g., methylumbelliferone, MUF, or L-3,4-dihydroxyphenylalanine, L-DOPA), linked to the substrate that the enzyme was able to hydrolyse or oxidase, reflecting potential degrading capacities of the community and may indicate its potential role on specific biogeochemical cycles. The main limitation of this method consists in detecting the potential enzyme activity of the microbial community, due to the fact that the artificial substrate is added at saturated concentration. Thus, the results indicate potential capacities but not real activities since for instance organic substrate concentration for the specific enzyme may exist in lower than saturation conditions in the environment. Here, this functional metric (especially EEAs linked to the degradation of labile organic matter) was positively correlated to the active prokaryote community fraction described by the RNA-based approach. Importantly, this finding underlines the relevance of the extracellular enzyme activities metric for the description of the ecosystem functions being related with the active microbial community inhabiting the streambed.

Similarly, among the molecular tools the DNA based approach was recognized as the most limited tool because it describes the global community without distinguishing between the active from non-active (or dead) cells. Also, in this thesis, the results obtained from the experimental approach brought some uncertainties regarding the utilization of the DNA community characterization, especially when studying a stress environmental factor, such as drought, on microbial communities (Chapter 2). Specifically, the weak variation of the bacterial community composition inhabiting the hyporheic sediment compared to that of the surface was questioned whether it was due to the fact that all DNA was analysed and thus probably including living and a large percentage of dead cells.

On the other hand, some consistencies appear when analysing both DNA and RNA. Notably, though the DNA and RNA prokaryote and eukaryote community's characterization run in the Chapter 3, several observations already described in Chapter 2 were confirmed. In particular, the wet and hyporheic bacterial communities were less variable than those inhabiting the surface sediment, independently from the drought treatment and the metabolic state. Also, RNA and DNA eukaryote characterization revealed variable eukaryote and fungal composition under the distinct hydrological phases, independently from the metabolic state.

Despite these positive confirmations given by the matrices comparison approach (Chapter 3), the extrapolation and interpretation of experimental results to natural intermittent ecosystems must be done with caution. Overall, the combination of different techniques (functional, structural and molecular) and different study approaches (laboratory or field) could be considered in order to better understand the microbial-mediated ecological processes occurring at the system level.

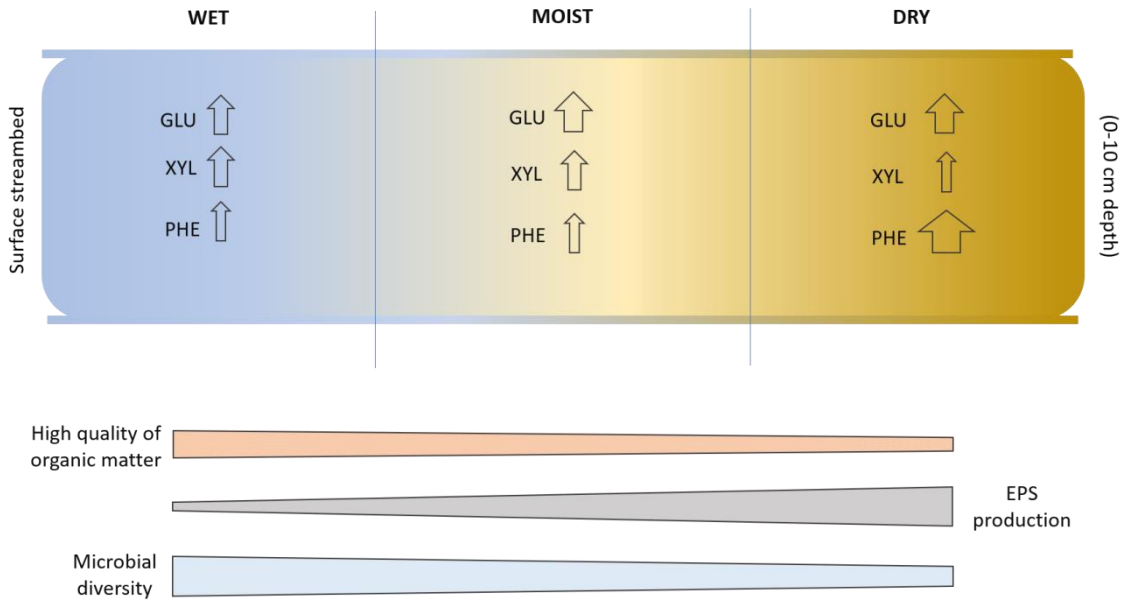


Figure 4. A conceptual model illustrating the main findings obtained from this Thesis. The acronyms GLU, XYL and PHE indicate the three extracellular enzyme activities (EEAs) of β-Glucosidase, β-Xylosidase and Phenol-oxidase, respectively. The acronym EPS states for extracellular polymeric substances. WET, MOIST and DRY indicate three distinct status of the superficial streambed. Microbial divestiy indicates the measures of Shannon-Wiener and Richness indices.

5.3 *Resistance to climate-anthropogenic hydrological changes; driving forces and responses at different scales:*

In this thesis, several driving forces affecting the streambed microbial community (functions, structure and composition) and its responses, co-occurring in parallel to hydrological changes were raised from the results obtained at different scales, such as microbial, habitat and catchment.

5.3.1 Microbial-scale: EPS production

Recent studies reported that extreme dry and rewetting stress resulted in similar mechanisms in soils and dry streambed microbial communities (Manzoni et al., 2012; Schimel, J., 2007; Zeglin, 2015). Among others, the increased production of extracellular polymeric substances (EPS) in dried sediments and soils (Flemming and Wingender, 2010; Or et al., 2007; Roberson and Firestone, 1992) is of particular interest, being substances produced by the biofilm microbiota (algae, bacteria, fungi), in order to preserve the humidity of the sediment and to store organic matter materials under drying process (Romaní et al., 2013; Sabater et al., 2016). For those reasons, these jelly substances were often hypothesized being a microbial strategy to cope with external harsh conditions, such as extreme desiccation (Ho et al., 2017; Romaní et al., 2013). Supporting this theory, the results from Chapters 1 and 2 described a sharp increase of EPS production in the surface sediment under prolonged dryness coupled with its abrupt decrease when the sediment was rewetted (e.g. punctual storms or final rewetting). Specifically, the relationship between the Actinobacteria class relative abundance and the EPS production resulted increasing during dry phase (Drought treatment) in the surface sediment, while alpha- and beta-proteobacteria relative abundances clearly decreased (Fig. S1). These findings gave reasons to correctly identify the EPS as strategic response against the desiccation, also supported by the evidence that this trend of production was mainly consistent in the driest surface sediment and not in the moister hyporheic sediment (Chapters 1 and 2).

5.3.2 *Microhabitat selection*

The habitat and micro-habitat characteristics are recognized as important modulators for the microbiota resistance and resilience to changes in the environmental conditions (Belmar et al., 2013; Hamilton et al., 2005; Romaní and Sabater, 20012). Several co-occurring factors a part from water scarcity can stress bacterial, archaeal, fungal and protozoa communities inhabiting the intermittent streambeds such as UV radiation, conductivity, temperature, oxygen (Augspurger et al., 2008; Risse-Buhl et al., 2012; Romaní et al., 2006b).

On the other hand, flow intermittency is known to determine a patchy distribution of habitats (such as lentic pools, leaves packs, moist and dry spots of sediment) which may contemporary act as natural filter, selecting for the most adapted species, and as refuge for the most sensitive ones (Datry et al., 2016; Lake, 2000; Larned et al., 2010). During the drying phase the microbiota would be distributed among these micro-habitats depending on the ability and characteristics of the distinct community to cope with desiccation and to disperse (e.g. from the sediment surface to the hyporheic and vice versa) between microhabitats (Barnard et al., 2013). Generally, the deeper hyporheic zone and the buried leaf packs within the streambed are considered important compartments and sources of humidity and nutrients, colonized by prokaryotes and eukaryotes as drying persist (Cornut et al., 2010; Febria et al., 2012; Mora-Gómez et al., 2016; Timoner et al., 2014a; Ylla et al., 2010). At habitat-scale the experimental results obtained from this thesis added important confirmations about the strategic refuge offered by the moister and oxygenated (because of desiccation) hyporheic zone, which allowed the microbial functional and structural resistance (Chapter 1). Also, the wet events (such as short summer precipitations) experimentally simulated, interrupting the perdurable dryness, promoted the microbial functional resilience and limited the alteration of the bacterial community composition (Chapters 1 and 2) most probably because of the increased water sediment content. Indeed, the water content stored in the streambed resulted in the major force influencing both the functions (Chapter 1) and the total and active community composition (Chapters 2 and 3). For these reasons, being the degree of sediment moisture essential for microbial community conservation, and varying under different conditions (e.g. degree of direct UV-radiation; physical slope; sediment granulometry, groundwater recharge and level) should be recognized as important factor to control when managing the intermittent rivers conservation.

5.3.3 *Catchment-scale: agricultural land-use and riparian vegetation development*

Up-scaling to the catchment level, agriculture land-use and deforestation derived to changes in the structure of soil microbial communities and their functions (Levine et al., 2011). Similarly, the mobilization of allochthonous materials imported from the surrounding agriculture watersheds during flooding and rewetting events can influence the heterotrophic productivity and respiration (Fierer et al., 2003b; Orchard and Cook, 1983; Timoner et al., 2012). Changes in the microbial biomass, diversity and activity are main consequences reported from agricultural land-use impact (Boëchat et al., 2014, 2011). On the other hand, the natural filter provided by a large forest land-use and preserved riparian vegetation associated to the streams were recognized of paramount importance because providing essential wildlife

habitat and allochthonous inputs of organisms and organic matter (e.g. leaves autumn fall) in the stream path. Furthermore, lateral exchanges of nutrients and organisms between the channel and the riparian vegetation are relevant functions of intermittent streams (Acuña et al., 2007; Steward et al., 2018, 2012). Accordingly, the multiple intermittent stream sites study conducted in this thesis, remarked that prokaryote community diversity was importantly reduced by the increase of the agricultural land-use at catchment scale and when reduced the riparian vegetation coverage (Chapter 4). Bearing in mind the multiple factors playing in the field studies, such as mixed effects of several natural biotic and abiotic factors contemporary acting and aware that this study consisted in a snapshot blocked in space and time, the findings obtained at catchment scale add importance for the preservation of the catchment when managing the intermittent freshwaters.

5.4 *Open questions and future trends*

In this thesis, prolonged drought responses in intermittent streambed associated microbial communities were studied through laboratory and field approaches. Despite there are promising findings that have come out from the extensive research conducted, some limitations in the methodologies preclude drawing more global conclusions about the observed trends. Here, we present a brief overview of these issues and present suggestions for future research to address them.

One limitation was the omission of the specific study of some communities, such as fungi and protozoa, which are also implicated in the overall streambed microbial response to drought stress. These groups are necessary for understanding the whole microbiota dynamics under environmental changes. More than the individual study of the fungal or protozoa responses under extended dry phase, knowledge about the specific microbial food web-interconnections are lacking in literature. Future studies in this direction would help refine our knowledge and determine the universality about microbiome trends under consistent drought stress, for instance, elucidating to what extent streambed microbial composition is modulated between the different groups and how these relationships differ under drought.

A significant hindrance in analysing the dry streambed microbiota is the methodology used to elucidate the effect on the associated communities. Relatively few studies combined functional, structural and taxonomical analyses, especially when more than one microbial group is considered. Association of molecular and functional/structural tools, used as proxy of the ecosystem processes, became essential for the next-generation studies, where the multiple-factors modelling is considered the first step to better analyse the changing environment. Future studies need to combine laboratory and field approaches in order to

draw meaningful and more reliable conclusions. For those studies, a proper data treatment such as that offered by multivariate and global models' analyses would be essential in support the numerical observations. Undoubtedly, the results from this thesis remarked also that time and space are relevant when comparing microbial responses from different stream sites. Larger time and space series coupled with specific characterization of the stream sites (at habitat and catchment scales) should be included in future research in order to better model the potential effects driven by increased drought conditions. Future studies also need to investigate whether the combination between the agricultural activity and the reduced riparian vegetation with the enlargement of the desiccation period could end up revealing additive negative effects, such as reduced microbial diversity and functions.

These suggestions could be considered as support for future investigations which bump into the increase threat of climate change that could irreversible change the streambed microbiota functions, and so increase the loss of freshwater ecosystems which in turn reduce the water security.

Life is short and potential studies infinite. We have a much better chance of accomplishing something significant when we follow our passionate interests and work in areas of deepest personal meaning.

Stephen Jay Gould

6. GENERAL CONCLUSIONS

Streambed microbes still have a lot to teach me.
Time and place for everything.

This section compiles the main conclusions of this thesis.

Chapter 1: Key role of streambed moisture and flash storms for microbial resistance and resilience to long- term drought

1. Microbial communities in surface sediment and leaf litter substrata were the most affected by the long-term drought as shown by the reduction of polysaccharide-degrading enzyme activities and litter decomposition rates. Also, in surface sediment, bacterial viability and algal biomass were promptly reduced due to (or during) long-term drought.
2. Greater use of recalcitrant compounds was observed under desiccation in the surface sediment and this was related to the reduced availability of labile organic matter sources and the prevalence of fungi over bacteria, the former being more resistant to drought. The production of extracellular polymeric substances increased with dryness, suggesting its potential role as adaptation of streambed biofilms to cope with water stress.
3. Hyporheic sediments appeared more resistant to long-term drought than surface sediments. This different response may be explained by a greater content of fine material in hyporheic sediments that permitted to better retain water content allowing fungi and bacteria to survive during droughts.
4. Punctual storms promoted resilience in microbial communities exposed to long-term drought during the rewetting phase as shown by the fast recovery of enzyme activities and bacterial viability in sediments and litter decomposition rates once rewetted.

Chapter 2: Distinct responses from bacterial, archaeal and fungal streambed communities to severe hydrological disturbances

1. Hydrological disturbances modified the archaeal community composition more than the bacterial one in streambed sediments, whereas fungi were the least affected microbial group.
2. The increase of certain classes' abundance in archaea (Thermoplasmata) and bacteria (Actinobacteria and Bacilli) revealed signs of transitioning from wet to a soil-like microbial community composition.
3. Water return (as sporadic storms and rewetting) led to larger shifts in the microbial community composition and diversity of surface sediments than those observed during the drying phase for the same type of community.
4. Microhabitat characteristics within the streambed, such as the greater capacity of the hyporheic zone in conserving moisture, serve the bacterial community as a refuge during severe hydrological disturbances with water stress.

Chapter 3: Total, active and functional streambed microbial diversity: comparing prokaryote and eukaryote responses to hydrological constrains

1. The prokaryote and eukaryote RNA diversity matrices were related to the extracellular enzyme activities suggesting that the functional shifts in organic matter decomposition were occurring in the active microbial community inhabiting streambed sediments subjected to altered hydrology.
2. Each aspect studied by the distinct diversity matrices (DNA and RNA approach) appeared complementary to assess communities' sensitivity to altered hydrology. Also, specific microbial dynamic description would be better described when several functional and molecular tools are considered.
3. The functional metrics, such as the Community Level Physiological Profiles and the Extracellular Enzyme Activities, confirmed their sensitivity to environmental changes.

Chapter 4: Multi-model assessment of hydrological history impact on microbial structural and functional responses in Mediterranean catchments

1. The microbial community composition and activity of surface sediments in 37 Mediterranean streams, sampled in wet conditions, was influenced by the specific hydrological history of each site. Contrarily, the sediment microbial community structure remained unchanged regardless of dry length antecedents.
2. Community composition of flowing sites was clearly distinct to those from intermittent sites, including short medium and long-dry phase sites.
3. Functional metrics in sediment microbial communities subjected to extended dry phase showed a progressive increase of recalcitrant carbon degradation activity.
4. Bacterial biomass and diversity from streambed surface sediments were mostly related to environmental features of the catchment (riparian vegetation coverage and agricultural land use) rather than to specific hydrological features. The overall maintenance of the microbial diversity under extreme dry streambed condition suggests reduced loss in the system functioning or potential reestablishment after water resumption.

REFERENCES

Aanderud, Z.T., Jones, S.E., Fierer, N., Lennon, J.T., 2015. Resuscitation of the rare biosphere contributes to pulses of ecosystem activity. Front. Microbiol. 6, 1–11. https://doi.org/10.3389/fmicb.2015.00024

Aanderud, Z.T., Vert, J.C., Lennon, J.T., Magnusson, T.W., Breakwell, D.P., Harker, A.R., 2016. Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. Front. Microbiol. 7, 1–13. https://doi.org/10.3389/fmicb.2016.00853

Abril, M., Muñoz, I., Menéndez, M., 2016. Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. Sci. Total Environ. 553, 330–339. https://doi.org/10.1016/j.scitotenv.2016.02.082

Acosta-Martínez, V., Cotton, J., Gardner, T., Moore-Kucera, J., Zak, J., Wester, D., Cox, S., 2014. Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. Appl. Soil Ecol. 84, 69–82. https://doi.org/10.1016/j.apsoil.2014.06.005

Acuña, V., Datry, T., Marshall, J., Barcelo, D., Dahm, C.N., Ginebreda, A., McGregor, G., Sabater, S., Tockner, K., Palmer, M.A., 2014. Why Should We Care About Temporary Waterways? Science (80-.). 343, 1080–1081. https://doi.org/10.1126/science.1246666

Acuña, V., Giorgi, A., Muñoz, I., Sabater, F., Sabater, S., 2007. Meteorological and riparian influences on organic matter dynamics in a forested Mediterranean stream. J. North Am. Benthol. Soc. 26, 54–69. https://doi.org/10.1899/0887-3593(2007)26[54:marioo]2.0.co;2

Adams, H.E., Crump, B.C., Kling, G.W., 2015. Isolating the effects of storm events on arctic aquatic bacteria: Temperature, nutrients, and community composition as controls on bacterial productivity. Front. Microbiol. 6, 1–14. https://doi.org/10.3389/fmicb.2015.00250

Allan, J.D., 2004. Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122

Allison, S.D., Goulden, M.L., 2017. Consequences of drought tolerance traits for microbial decomposition in the DEMENT model. Soil Biol. Biochem. 107, 104–113. https://doi.org/10.1016/j.soilbio.2017.01.001

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. 105, 11512–11519. https://doi.org/10.1073/pnas.0801925105

Amalfitano, S., Fazi, S., 2008. Recovery and quantification of bacterial cells associated with streambed sediments. J. Microbiol. Methods 75, 237–243. https://doi.org/10.1016/j.mimet.2008.06.004

Angel, R., Pasternak, Z., Soares, M.I.M., Conrad, R., Gillor, O., 2013. Active and total prokaryotic communities in dryland soils. FEMS Microbiol. Ecol. 86, 130–138. https://doi.org/10.1111/1574-6941.12155

Arce, M.I., Mendoza-Lera, C., Almagro, M., Catalán, N., Romaní, A.M., Martí, E., Gómez, R., Bernal, S., Foulquier, A., Mutz, M., Marcé, R., Zoppini, A., Gionchetta, G., Weigelhofer, G., del Campo, R., Robinson, C.T., Gilmer, A., Rulik, M., Obrador, B., Shumilova, O., Zlatanović, S., Arnon, S., Baldrian, P., Singer, G., Datry, T., Skoulikidis, N., Tietjen, B., von Schiller, D., 2019. A conceptual framework for understanding the biogeochemistry of dry riverbeds through the lens of soil science. Earth-Science Rev. 188, 441–453. https://doi.org/10.1016/j.earscirev.2018.12.001

Argyroudi, A., Chatzinikolaou, Y., Poirazidis, K., Lazaridou, M., 2009. Do intermittent and ephemeral mediterranean rivers belong to the same river type? Aquat. Ecol. 43, 465–476. https://doi.org/10.1007/s10452-008-9176-9

Arias-Real, R., Menéndez, M., Abril, M., Oliva, F., Muñoz, I., 2018. Quality and quantity of leaf litter: Both are important for feeding preferences and growth of an aquatic shredder. PLoS One 13, e0208272. https://doi.org/10.1371/journal.pone.0208272

Artigas, J., Romaní, A.M., Gaudes, A., Muñoz, I., and Sabater, S. (2009). Organic matter availability structures microbial biomass and activity in a Mediterranean river. Freshwat. Biol. 54, 2025–2036. doi:10.1111/j.1365-2427.2008.02140.x

Artigas, J., Gaudes, A., Muñoz, I., Romaní, A.M. and Sabater, S. (2011). Fungal and Bacterial Colonization of Submerged Leaf Litter in a Mediterranean Stream. Internat. Rev. Hydrobiol Internat. Rev. Hydrobiol. 96/3. 221–234. doi:10.1002/iroh.201111355

Aslam, S.N., Dumbrell, A.J., Sabir, J.S., Mutwakil, M.H.Z., Baeshen, M.M.N., Abo-Aba, S.E.M., Clark, D.R., Yates, S.A., Baeshen, N.A., Underwood, G.J.C., McGenity, T.J., 2016. Soil compartment is a major determinant of the impact of simulated rainfall on desert microbiota. Environ. Microbiol. 18, 5048–5062. https://doi.org/10.1111/1462-2920.13474

Attermeyer, K., Premke, K., Hornick, T., Hilt, S., Grossart, H.P., 2013. Ecosystem-level studies of terrestrial carbon reveal contrasting bacterial metabolism in different aquatic habitats. Ecology 94, 2754–2766. https://doi.org/10.1890/13-0420.1

Augspurger, C., Gleixner, G., Kramer, C., Küsel, K., 2008. Tracking carbon flow in a 2-week-old and 6-week-old stream biofilm food web. Limnol. Oceanogr. 53, 642–650. https://doi.org/10.4319/lo.2008.53.2.0642

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141, 221–35. https://doi.org/10.1007/s00442-004-1519-1

Baldwin, D.S., Mitchell, A.M., 2002. The effects of drying and re-flooding on the sediment and soil nutrient dynamics

of lowland river–floodplain systems: a synthesis. Regul. Rivers Res. Manag. 16, 457–467. https://doi.org/https://doi.org/10.1002/1099-1646(200009/10)16:5<457::AID-RRR597>3.0.CO;2-B
Baldy, V., Gessner, M.O., 1997. Towards a budget of leaf litter decomposition in a first order woodland stream. Comptes Rendus l´Académie des Sci. Ser. III 747–758.
Bärlocher, F., Nikolcheva, L.G., Wilson, K.P., Williams, D.D., 2006. Fungi in the hyporheic zone of a springbrook. Microb. Ecol. 52, 708–715. https://doi.org/10.1007/s00248-006-9102-4
Barnard, R.L., Osborne, C.A., Firestone, M.K., 2014. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. ISME J. 9, 946–957. https://doi.org/10.1038/ismej.2014.192
Barnard, R.L., Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J. 7, 2229–2241. https://doi.org/10.1038/ismej.2013.104
Barthès, A., Ten-Hage, L., Lamy, A., Rols, J.L., Leflaive, J., 2015. Resilience of AggregatedMicrobial Communities Subjected to Drought—Small-Scale Studies. Microb. Ecol. 9–20. https://doi.org/10.1007/s00248-014-0532-0
Baschien, C., Tsui, C.K.M., Gulis, V., Szewzyk, U., Marvanová, L., 2013. The molecular phylogeny of aquatic hyphomycetes with affinity to the Leotiomycetes. Fungal Biol. 117, 660–672. https://doi.org/10.1016/j.funbio.2013.07.004
Battin, T.J., 2000. Hydrodynamics is a major determinant of streambed activity: From the sediment to the reach scale. Limnol. Oceanogr. 45, 1308–1319. https://doi.org/https://doi.org/10.4319/lo.2000.45.6.1308
Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14, 251–263. https://doi.org/10.1038/nrmicro.2016.15
Battin, T.J., Kaplan, L.A., Newbold, J.D., Hansen, C.M.E., 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature 426, 439–442. https://doi.org/10.1038/nature02152
Battin, T.J., Sengschmitt, D., 1999. Linking sediment biofilms, hydrodynamics, and river bed clogging: Evidence from a large river. Microb. Ecol. 37, 185–196. https://doi.org/10.1007/s002489900142
Belmar, O., Bruno, D., Martínez-Capel, F., Barquín, J., Velasco, J., 2013. Effects of flow regime alteration on fluvial habitats and riparian quality in a semiarid Mediterranean basin. Ecol. Indic. 30, 52–64. https://doi.org/10.1016/j.ecolind.2013.01.042
Bengtsson, M.M., Wagner, K., Schwab, C., Urich, T., Battin, T.J., 2018. Light availability impacts structure and function of phototrophic stream biofilms across domains and trophic levels. Mol. Ecol. 27, 2913–2925. https://doi.org/10.1111/mec.14696
Benstead, J.P., Leigh, D.S., 2012. An expanded role for river networks. Nat. Geosci. 5, 678–679. https://doi.org/10.1038/ngeo1593
Bérard, A., Bouchet, T., Sévenier, G., Pablo, A.L., Gros, R., 2011. Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. Eur. J. Soil Biol. 47, 333–342. https://doi.org/10.1016/j.ejsobi.2011.08.004
Berggren, M., Giorgio, P. a, 2015. Distinct patterns of microbial metabolism associated to riverine dissolved organic carbon of different source and quality. J. Geophys. Res. Biogeosciences 120, 989–999. https://doi.org/10.1002/2015JG002963
Berggren, M., Laudon, H., Jansson, M., 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters. Global Biogeochem. Cycles 21, 1–9. https://doi.org/10.1029/2006GB002844
Besemer, K., Singer, G., Quince, C., Bertuzzo, E., Sloan, W., Battin, T.J., 2013. Headwaters are critical reservoirs of microbial diversity for fluvial networks. Proc. Biol. Sci. 280, 20131760. https://doi.org/10.1098/rspb.2013.1760
Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil X, 9–10.
Birch, H.F., Griffiths, E., 1961. Microbiological changes in freshly moistened soil. Nature 189, 424.
Blazewicz, S.J., Barnard, R.L., Daly, R.A., Firestone, M.K., 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: Limitations and uses. ISME J. 7, 2061–2068. https://doi.org/10.1038/ismej.2013.102
Blazewicz, S.J., Schwartz, E., Firestone, M.K., 2014. Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. Ecology 95, 1162–1172. https://doi.org/10.1890/13-1031.1
Boëchat, I.G., Krüger, A., Chaves, R.C., Graeber, D., Gücker, B., 2014. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. Sci. Total Environ. 482–483, 62–70. https://doi.org/10.1016/j.scitotenv.2014.02.111
Boëchat, I.G., Krüger, A., Giani, A., Figueredo, C.C., Gücker, B., 2011. Agricultural land-use affects the nutritional quality of stream microbial communities. FEMS Microbiol. Ecol. 77, 568–576. https://doi.org/10.1111/j.1574-6941.2011.01137.x
Bonada, N., Resh, V.H., 2013. Mediterranean-climate streams and rivers: Geographically separated but ecologically comparable freshwater systems. Hydrobiologia 719, 1–29. https://doi.org/10.1007/s10750-013-1634-2
Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob. Chang. Biol. 15, 808–824. https://doi.org/10.1111/j.1365-2486.2008.01681.x
Botter, G., Basso, S., Rodriguez-Iturbe, I., Rinaldo, A., 2013. Resilience of river flow regimes. Proc. Natl. Acad. Sci. 110, 12925–12930. https://doi.org/10.1073/pnas.1311920110
Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H., Valett, H.M., 1998. The functional significance of the

hyporheic zone in streams and rivers. Annu. Rev. Ecol. Syst. 29, 59–81. https://doi.org/10.1146/annurev.ecolsys.29.1.59
Bouskill, N.J., Lim, H.C., Borglin, S., Salve, R., Wood, T.E., Silver, W.L., Brodie, E.L., 2013. Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. ISME J. 7, 384–394. https://doi.org/10.1038/ismej.2012.113
Brablcová, L., I. Buriánková, P. Badurová, and M. Rulík. 2013. The phylogenetic structure of microbial biofilms and free-living bacteria in a small stream. Folia Microbiol. (Praha). 58: 235–43.
Brandt, F.B., Martinson, G.O., Pommerenke, B., Pump, J., Conrad, R., 2015. Drying effects on archaeal community composition and methanogenesis in bromeliad tanks. FEMS Microbiol. Ecol. 91, 1–10. https://doi.org/10.1093/femsec/iu021
Bruder, A., Chauvet, E., Gessner, M.O., 2011. Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency. Funct. Ecol. 25, 1269–1277. https://doi.org/10.1111/j.1365-2435.2011.01903.x
Bruno, D., Gutiérrez-Cánovas, C., Sánchez-Fernández, D., Velasco, J., Nilsson, C., 2016. Impacts of environmental filters on functional redundancy in riparian vegetation. J. Appl. Ecol. 53, 846–855. https://doi.org/10.1111/1365-2664.12619
Bunn, S.E., Thoms, M.C., Hamilton, S.K., Capon, S.J., 2006. Flow variability in dryland rivers: Boom, bust and the bits in between. River Res. Appl. 22, 179–186. https://doi.org/10.1002/rra.904
Buriankova, Iva, M.R., 2013. Identification of methanogenic archaea in the hyporheic sediment of Sitka stream. PLoS One 8, 1–10. https://doi.org/10.1371/journal.pone.0080804
Burnham, K. P., & Anderson, D.R., 2002. Model Selection and Multimodel Inference, Second edi. ed. Springer.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58, 216–234. https://doi.org/10.1016/j.soilbio.2012.11.009
Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69, 3593–9. https://doi.org/10.1128/AEM.69.6.3593
Canhoto, C., Gonçalves, A.L., Bärlocher, F., 2016. Biology and ecological functions of aquatic hyphomycetes in a warming climate. Fungal Ecol. 19, 201–218. https://doi.org/10.1016/j.funeco.2015.09.011
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108, 4516–4522. https://doi.org/10.1073/pnas.1000080107
Casas-Ruiz, J.P., Tittel, J., von Schiller, D., Catalán, N., Obrador, B., Gómez-Gener, L., Zwirnmann, E., Sabater, S., Marcé, R., 2016. Drought-induced discontinuities in the source and degradation of dissolved organic matter in a Mediterranean river. Biogeochemistry 127, 125–139. https://doi.org/10.1007/s10533-015-0173-5
Cherifi, O., Loudiki, M., 1999. Flood transport of dissolved and suspended matter in the El Abid river basin (Morocco). Hydrobiologia 410, 287–294. https://doi.org/10.1023/A:1003898301704
Choi, K.H., Dobbs, F.C., 1999. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. J. Microbiol. Methods 36, 203–213. https://doi.org/10.1016/S0167-7012(99)00034-2
Cipriani, T., Tilmant, F., Branger, F., Sauquet, E., Datry, T., 2014. Impact of climate change on aquatic ecosystems along the Asse River network 2014, 2050.
Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal Database Project: Data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42, 633–642. https://doi.org/10.1093/nar/gkt1244
Collins, R., Kristensen, P., Thyssen, N., 2009. Water resources across Europe - confronting water scarcity and drought. EEA Rep. 60. https://doi.org/10.2800/16803
Compte-Port, S., Subirats, J., Fillol, M., Sànchez-Melsió, A., Marcé, R., Rivas-Ruiz, P., Rosell-Melé, A., Borrego, C.M., 2017. Abundance and Co-Distribution of Widespread Marine Archaeal Lineages in Surface Sediments of Freshwater Water Bodies across the Iberian Peninsula. Microb. Ecol. 74, 776–787. https://doi.org/10.1007/s00248-017-0989-8
Cornut, J., Elger, A., Lambrigot, D., Marmonier, P., Chauvet, E., 2010. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshw. Biol. 55, 2541–2556. https://doi.org/10.1111/j.1365-2427.2010.02483.x
Corti, R., Datry, T., Drummond, L., Larned, S.T., 2011. Natural variation in immersion and emersion affects breakdown and invertebrate colonization of leaf litter in a temporary river. Aquat. Sci. 73, 537–550. https://doi.org/10.1007/s00027-011-0216-5
Costigan, K.H., Jaeger, K.L., Goss, C.W., Fritz, K.M., Goebel, P.C., 2016. Understanding controls on flow permanence in intermittent rivers to aid ecological research: integrating meteorology, geology and land cover. Ecohydrology 9, 1141–1153. https://doi.org/10.1002/eco.1712
Datry, T., Bonada, N., Boulton, A.J., 2017a. Intermittent Rivers and Ephemeral Streams - Ecology and Management, Academic P. ed, Elsevier Inc. All rights reserved.

Datry, T., Boulton, A.J., Bonada, N., Fritz, K., Leigh, C., Sauquet, E., Tockner, K., Hugueny, B., Dahm, C.N., 2017b. Flow intermittence and ecosystem services in rivers of the Anthropocene. J. Appl. Ecol. 1–12. https://doi.org/10.1111/1365-2664.12941
Datry, T., Fritz, K., Leigh, C., 2016. Challenges, developments and perspectives in intermittent river ecology. Freshw. Biol. 61, 1171–1180. https://doi.org/10.1111/fwb.12789
Datry, T., Larned, S.T., 2008. River flow controls ecological processes and invertebrate assemblages in subsurface flowpaths of an ephemeral river reach. Can. J. Fish. Aquat. Sci. 65, 1532–1544. https://doi.org/10.1139/f08-075
Datry, T., Larned, S.T., Tockner, K., 2014. Intermittent rivers: A challenge for freshwater ecology. Bioscience 64, 229–235. https://doi.org/10.1093/biosci/bit027
De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29, 795–811. https://doi.org/10.1016/j.femsre.2004.11.005
de Vries, F.T., Shade, A., 2013. Controls on soil microbial community stability under climate change. Front. Microbiol. 4, 1–16. https://doi.org/10.3389/fmicb.2013.00265
Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry - wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Tillage Res. 33, 1599–1611. https://doi.org/10.1016/S0038-0717(01)00076-1
Dexter, A.R., 1988. Advances in characterization of soil structure. Soil Tillage Res. 11, 199–238. https://doi.org/10.1016/0167-1987(88)90002-5
Dickie, I.A., Wood, J.R., Holdaway, R.J., Orwin, K.H., Bonner, K.I., 2015. Soil microbial community structure explains the resistance of respiration to a dry-rewet cycle, but not soil functioning under static conditions. Funct. Ecol. 30, 1430–1439. https://doi.org/10.1111/1365-2435.12610
Dieter, D., von Schiller, D., García-Roger, E.M., Sánchez-Montoya, M.M., Gómez, R., Mora-Gómez, J., Sangiorgio, F., Gelbrecht, J., Tockner, K., 2011. Preconditioning effects of intermittent stream flow on leaf litter decomposition. Aquat. Sci. 73, 599–609. https://doi.org/10.1007/s00027-011-0231-6
Dlott, G., Maul, J.E., Buyer, J., Yarwood, S., 2015. Microbial rRNA: RDNA gene ratios may be unexpectedly low due to extracellular DNA preservation in soils. J. Microbiol. Methods 115, 112–120. https://doi.org/10.1016/j.mimet.2015.05.027
Döll, P., Schmied, H.M., 2012. How is the impact of climate change on river flow regimes related to the impact on mean annual runoff? A global-scale analysis. Environ. Res. Lett. 7. https://doi.org/10.1088/1748-9326/7/1/014037
Drenovsky, R.E., Steenwerth, K.L., Jackson, L.E., Scow, K.M., 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Glob. Ecol. Biogeogr. 19, 27–39. https://doi.org/10.1111/j.1466-8238.2009.00486.x
Duarte, S., Mora-Gómez, J., Romaní, A.M., Cássio, F., Pascoal, C., 2017. Responses of microbial decomposers to drought in streams may depend on the environmental context. Environ. Microbiol. Rep. 9, 756–765. https://doi.org/10.1111/1758-2229.12592
Duran, X., Picó, M., Reales, L., 2017. Climate change in Catalonia: Executive summary of the third report on climate change in Catalonia. Institute of Catalan Studies and the Government of Catalonia.
Edgar, R.C., Flyvbjerg, H., 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31, 3476–3482. https://doi.org/10.1093/bioinformatics/btv401
Elosegi, A., Díez, J., Mutz, M., 2010. Effects of hydromorphological integrity on biodiversity and functioning of river ecosystems. Hydrobiologia 657, 199–215. https://doi.org/10.1007/s10750-009-0083-4
Estrela, T., Perez-Martin, M. a, Vargas, E., 2012. Impacts of climate change on water resources in Spain. Hydrol. Sci. Journal-Journal Des Sci. Hydrol. 57, 1154–1167. https://doi.org/10.1080/02626667.2012.702213
Evans, S.E., Wallenstein, M.D., 2014. Climate change alters ecological strategies of soil bacteria. Ecol. Lett. 17, 155–164. https://doi.org/10.1111/ele.12206
Evans, S.E., Wallenstein, M.D., 2012. Soil microbial community response to drying and rewetting stress: Does historical precipitation regime matter? Biogeochemistry 109, 101–116. https://doi.org/10.1007/s10533-011-9638-3
Evans, S.E., Wallenstein, M.D., Burke, I.C., 2014. Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought. Ecology 95, 110–122. https://doi.org/10.1890/13-0500.1
Fabian, J., Zlatanovic, S., Mutz, M., Premke, K., 2017. Fungal–bacterial dynamics and their contribution to terrigenous carbon turnover in relation to organic matter quality. ISME J. 11, 415–425. https://doi.org/10.1038/ismej.2016.131
Fazi, S., Amalfitano, S., Piccini, C., Zoppini, A., Puddu, A., Pernthaler, J., 2008. Colonization of overlaying water by bacteria from dry river sediments. Environ. Microbiol. 10, 2760–2772. https://doi.org/10.1111/j.1462-2920.2008.01695.x
Fazi, S., Vázquez, E., Casamayor, E.O., Amalfitano, S., Butturini, A., 2013. Stream Hydrological Fragmentation Drives Bacterioplankton Community Composition. PLoS One 8. https://doi.org/10.1371/journal.pone.0064109
Febria, C.M., Beddoes, P., Fulthorpe, R.R., Williams, D.D., 2012. Bacterial community dynamics in the hyporheic zone of an intermittent stream. ISME J. 6, 1078–1088. https://doi.org/10.1038/ismej.2011.173

Febria, C.M., Hosen, J.D., Crump, B.C., Palmer, M.A., Williams, D.D., 2015. Microbial responses to changes in flow status in temporary headwater streams: a cross-system comparison. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.00522
Feld, C.K., Segurado, P., Gutiérrez-Cánovas, C., 2016. Analysing the impact of multiple stressors in aquatic biomonitoring data: A 'cookbook' with applications in R. Sci. Total Environ. 573, 1320–1339. https://doi.org/10.1016/j.scitotenv.2016.06.243
Fierer, N., Schimel, J.P., 2003. A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil. Soil Sci. Soc. Am. J. 67, 798. https://doi.org/10.2136/sssaj2003.0798
Fierer, N., Schimel, J.P., Holden, P.A., 2003a. Influence of drying-rewetting frequency on soil bacterial community structure. Microb. Ecol. 45, 63–71. https://doi.org/10.1007/s00248-002-1007-2
Fierer, N., Schimel, J.P., Holden, P.A., 2003b. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176. https://doi.org/10.1016/S0038-0717(02)00251-1
Findlay, S., 2010. Stream microbial ecology. J. North Am. Benthol. Soc. 29, 170–181. https://doi.org/10.1899/09-023.1
Finlay, B.J., Esteban, G.F., 2009. Oxygen sensing drives predictable migrations in a microbial community. Environ. Microbiol. 11, 81–85. https://doi.org/10.1111/j.1462-2920.2008.01742.x
Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575. https://doi.org/10.1038/nrmicro.2016.94
Flemming, H., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623–33. https://doi.org/10.1038/nrmicro2415
Floch, C., Chevremont, A.C., Joanico, K., Capowiez, Y., Criquet, S., 2011. Indicators of pesticide contamination: Soil enzyme compared to functional diversity of bacterial communities via Biolog® Ecoplates. Eur. J. Soil Biol. 47, 256–263. https://doi.org/10.1016/j.ejsobi.2011.05.007
Foody, G.M., Ghoneim, E.M., Arnell, N.W., 2004. Predicting locations sensitive to flash flooding in an arid environment. J. Hydrol. 292, 48–58. https://doi.org/10.1016/j.jhydrol.2003.12.045
Fossati, J., Pautou, G., Peltier, J.P., 1999. Water as resource and disturbance for wadi vegetation in a hyperarid area (Wadi Sannur, Eastern Desert, Egypt). J. Arid Environ. 43, 63–77. https://doi.org/10.1006/jare.1999.0526
Foulquier, A., Artigas, J., Pesce, S., Datry, T., 2015. Drying responses of microbial litter decomposition and associated fungal and bacterial communities are not affected by emersion frequency. Freshw. Sci. 34, 1233–1244. https://doi.org/10.1086/682060
Frank, Dorothea, Reichstein, M., Bahn, M., Thonicke, K., Frank, David, Mahecha, M.D., Smith, P., van der Velde, M., Vicca, S., Babst, F., Beer, C., Buchmann, N., Canadell, J.G., Ciais, P., Cramer, W., Ibrom, A., Miglietta, F., Poulter, B., Rammig, A., Seneviratne, S.I., Walz, A., Wattenbach, M., Zavala, M.A., Zscheischler, J., 2015. Effects of climate extremes on the terrestrial carbon cycle: Concepts, processes and potential future impacts. Glob. Chang. Biol. 21, 2861–2880. https://doi.org/10.1111/gcb.12916
Freese, H.M., Karsten, U., Schumann, R., 2006. Bacterial abundance, activity, and viability in the eutrophic River Warnow, northeast Germany. Microb. Ecol. 51, 117–127. https://doi.org/10.1007/s00248-005-0091-5
Freixa, A., Ejarque, E., Crognale, S., Amalfitano, S., Fazi, S., Butturini, A., Romaní, A.M., 2016. Sediment microbial communities rely on different dissolved organic matter sources along a Mediterranean river continuum. Limnol. Oceanogr. 61. https://doi.org/10.1002/lno.10308
Freixa, A., Rubol, S., Carles-Brangarí, A., Fernàndez-Garcia, D., Butturini, A., Sanchez-Vila, X., Romaní, A.M., 2015. The effects of sediment depth and oxygen concentration on the use of organic matter: An experimental study using an infiltration sediment tank. Sci. Total Environ. 540, 20–31. https://doi.org/10.1016/j.scitotenv.2015.04.007
Frossard, A., Gerull, L., Mutz, M., Gessner, M.O., 2012. Disconnect of microbial structure and function: enzyme activities and bacterial communities in nascent stream corridors. ISME J. 6, 680–691. https://doi.org/10.1038/ismej.2011.134
Frossard, A., Ramond, J.-B., Seely, M., Cowan, D. a., 2015. Water regime history drives responses of soil Namib Desert microbial communities to wetting events. Sci. Rep. 5, 1–13. https://doi.org/10.1038/srep12263
Gallo, E.L., Lohse, K.A., Ferlin, C.M., Meixner, T., Brooks, P.D., 2014. Physical and biological controls on trace gas fluxes in semi-arid urban ephemeral waterways. Biogeochemistry 121, 189–207. https://doi.org/10.1007/s10533-013-9927-0
Gao, X., Olapade, O.A., Leff, L.G., 2005. Comparison of benthic bacterial community composition in nine streams. Aquat. Microb. Ecol. 40, 51–60. https://doi.org/10.3354/ame040051
Gessner, M.O., Chauvet, E., 1994. Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75, 1807–1817. https://doi.org/10.2307/1939639
Gessner, M.O., Schmitt, A.L., 1996. Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi. Appl. Environ. Microbiol. 62, 415–419. https://doi.org/December, 21 2012
Ghiorse, WC, Wilson, J.T., 1988. Microbial Ecology of the Terrestrial Subsurface. Adv. Appl. Microbiol. 33, 107–172. https://doi.org/https://doi.org/10.1016/S0065-2164(08)70206-5
Giannakopoulos, C., Le Sager, P., Bindi, M., Moriondo, M., Kostopoulou, E., Goodess, C.M., 2009. Climatic changes and associated impacts in the Mediterranean resulting from a 2 °C global warming. Glob. Planet. Change 68,

209–224. https://doi.org/10.1016/j.gloplacha.2009.06.001
Gibbons, S.M., Jones, E., Bearquiver, A., Blackwolf, F., Roundstone, W., Scott, N., Hooker, J., Madsen, R., Coleman, M.L., Gilbert, J.A., 2014. Human and environmental impacts on river sediment microbial communities. PLoS One 9, 1–9. https://doi.org/10.1371/journal.pone.0097435
Gieswein, A., Hering, D., Feld, C.K., 2017. Additive effects prevail: The response of biota to multiple stressors in an intensively monitored watershed. Sci. Total Environ. 593–594, 27–35. https://doi.org/10.1016/j.scitotenv.2017.03.116
Gionchetta, G., Oliva, F., Menéndez, M., Lopez Laseras, P., Romaní, A.M., 2019. Key role of streambed moisture and flash storms for microbial resistance and resilience to long-term drought. Freshw. Biol. 64, 306–322. https://doi.org/10.1111/fwb.13218
Gómez-Gener, L., Obrador, B., Marcé, R., Acuña, V., Catalán, N., Casas-Ruiz, J.P., Sabater, S., Muñoz, I., von Schiller, D., 2016. When Water Vanishes: Magnitude and Regulation of Carbon Dioxide Emissions from Dry Temporary Streams. Ecosystems 19, 710–723. https://doi.org/10.1007/s10021-016-9963-4
Gómez-Gener, L., Obrador, B., von Schiller, D., Marcé, R., Casas-Ruiz, J.P., Proia, L., Acuña, V., Catalán, N., Muñoz, I., Koschorreck, M., 2015. Hot spots for carbon emissions from Mediterranean fluvial networks during summer drought. Biogeochemistry 125, 409–426. https://doi.org/10.1007/s10533-015-0139-7
Gómez, R., Arce, I.M., Sánchez, J.J., del Mar Sánchez-Montoya, M., 2012. The effects of drying on sediment nitrogen content in a Mediterranean intermittent stream: A microcosms study. Hydrobiologia 679, 43–59. https://doi.org/10.1007/s10750-011-0854-6
Gomi, T., Sidle, R.C., Richardson, J.S., 2002. Understanding Processes and Downstream Linkages of Headwater Systems. Bioscience 52, 905. https://doi.org/10.1641/0006-3568(2002)052[0905:upadlo]2.0.co;2
Gornall, J., Betts, R., Burke, E., Clark, R., Camp, J., Willett, K., Wiltshire, A., 2010. Implications of climate change for agricultural productivity in the early twenty-first century. Philos. Trans. R. Soc. B Biol. Sci. 365, 2973–2989. https://doi.org/10.1098/rstb.2010.0158
Görres, J.H., Savin, M.C., Neher, D.A., Weicht, T.R., Amador, J.A., 1999. Grazing in a porous environment: 1. The effect of soil pore structure on C and N mineralization. Plant Soil 212, 75–83. https://doi.org/10.1023/A:1004694202862
Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37, 112–129. https://doi.org/10.1111/j.1574-6976.2012.00343.x
Grimm, N.B., Gergel, S.E., McDowell, W.H., Boyer, E.W., Dent, C.L., Groffman, P., Hart, S.C., Harvey, J., Johnston, C., Mayorga, E., McClain, M.E., Pinay, G., 2003. Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. Oecologia 137, 485–501. https://doi.org/10.1007/s00442-003-1382-5
Grueber, C.E., Nakagawa, S., Laws, R.J., Jamieson, I.G., 2011. Multimodel inference in ecology and evolution: Challenges and solutions. J. Evol. Biol. 24, 699–711. https://doi.org/10.1111/j.1420-9101.2010.02210.x
Haggerty, R., Argerich, A., Martí, E., 2010. Development of a “smart” tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system. Water Resour. Res. 46, 1–10. https://doi.org/10.1029/2007WR006670
Hahn, M., 2002. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol. 35, 113–121. https://doi.org/10.1016/s0168-6496(00)00098-2
Hakenkamp, C.C., Morin, A., 2000. The importance of meiofauna to lotic ecosystem functioning. Freshw. Biol. 44, 165–175. https://doi.org/10.1046/j.1365-2427.2000.00589.x
Hamilton, S.K., Bunn, S.E., Thoms, M.C., Marshall, J.C., 2005. Persistence of aquatic refugia between flow pulses in a dryland river system (Cooper Creek, Australia). Limnol. Oceanogr. 50, 743–754. https://doi.org/10.4319/lo.2005.50.3.0743
Harms, T.K., Grimm, N.B., 2012. Responses of trace gases to hydrologic pulses in desert floodplains. J. Geophys. Res. Biogeosciences 117, 1–14. https://doi.org/10.1029/2011JG001775
Hassan, M.A., Egozi, R., 2001. Impact of wastewater discharge on the channel morphology of ephemeral streams. Earth Surf. Process. Landforms 26, 1285–1302. https://doi.org/10.1002/esp.273
Henry, H.A.L., 2013. Reprint of “Soil extracellular enzyme dynamics in a changing climate.” Soil Biol. Biochem. 56, 53–59. https://doi.org/10.1016/j.soilbio.2012.10.022
Ho, A., Di Lonardo, D.P., Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol. Ecol. 93, 1–14. https://doi.org/10.1093/femsec/fix006
Hoerling, M., Eischeid, J., Perlwitz, J., Quan, X., Zhang, T., Pegion, P., 2012. On the increased frequency of mediterranean drought. J. Clim. 25, 2146–2161. https://doi.org/10.1175/JCLI-D-11-00296.1
Holmes, N.T.H., 1999. Recovery of headwater stream flora following the 1989-1992 groundwater drought. Hydrol. Process. 13, 341–354. https://doi.org/10.1002/(SICI)1099-1085(19990228)13:3<341::AID-HYP742>3.0.CO;2-L
Humphries, P., Baldwin, D.S., 2003. Drought and aquatic ecosystems: an introduction. Freshw. Biol. 48, 1141–1146. https://doi.org/10.1002/9781444341812
IPCC, 2014. Part A: Global and Sectoral Aspects. (Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change). Clim. Chang. 2014 Impacts, Adapt. Vulnerability. 1132. https://doi.org/10.1017/CBO9781107415324.004
Itoh, T., Yoshikawa, N., Takashina, T., 2007. Thermogymnomonas acidicola gen. nov., sp. nov., a novel thermoacidophilic, cell wall-less archaeon in order Thermoplasmatales, isolated from a solfataric soil in

Hakone, Japan. Int. J. Syst. Evol. Microbiol. 57, 2557–2561. https://doi.org/10.1099/ijs.0.65203-0
Izbicki, J.A., 2007. Physical and temporal isolation of mountain headwater streams in the western Mojave Desert, Southern California. J. Am. Water Resour. Assoc. 43, 26–40. https://doi.org/10.1111/j.1752-1688.2007.00004.x
Jacobson, P.J., Jacobson, K.M., Angermeier, P.L., Cherry, D.S., 2000. Variation in material transport and water chemistry along a large ephemeral river in the Namib Desert. Freshw. Biol. 44, 481–491. https://doi.org/10.1046/j.1365-2427.2000.00604.x
Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. und Physiol. der Pflanz. 167, 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3
Jeraldo, P., Kalari, K., Chen, X., Bhavsar, J., Mangalam, A., White, B., Nelson, H., Kocher, J.P., Chia, N., 2014. IM-TORNADO: A tool for comparison of 16S reads from paired-end libraries. PLoS One 9, 1–19. https://doi.org/10.1371/journal.pone.0114804
Jessup, C.M., Kassen, R., Forde, S.E., Kerr, B., Buckling, A., Rainey, P.B., Bohannan, B.J.M., 2004. Big questions, small worlds: Microbial model systems in ecology. Trends Ecol. Evol. 19, 189–197. https://doi.org/10.1016/j.tree.2004.01.008
Jones, S.E., Lennon, J.T., 2010. Dormancy contributes to the maintenance of microbial diversity. Proc. Natl. Acad. Sci. 107, 5881–5886. https://doi.org/10.1073/pnas.0912765107
Jousset, A., 2012. Ecological and evolutive implications of bacterial defences against predators. Environ. Microbiol. 14, 1830–1843. https://doi.org/10.1111/j.1462-2920.2011.02627.x
Jürgens, K., Pernthaler, J., Schalla, S., Amann, R., 1999. Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl. Environ. Microbiol. 65, 1241–1250. https://doi.org/PMC91171
Kallimanis, A., Frillingos, S., Drainas, C., Koukkou, A.I., 2007. Taxonomic identification, phenanthrene uptake activity, and membrane lipid alterations of the PAH degrading Arthrobacter sp. strain Sphe3. Appl. Microbiol. Biotechnol. 76, 709–717. https://doi.org/10.1007/s00253-007-1036-3
Kathol, M., Fischer, H., Weitere, M., 2011. Contribution of biofilm-dwelling consumers to pelagic-benthic coupling in a large river. Freshw. Biol. 56, 1160–1172. https://doi.org/10.1111/j.1365-2427.2010.02561.x
Kerou, M., Offre, P., Valledor, L., Abby, S.S., Melcher, M., Nagler, M., Weckwerth, W., Schleper, C., 2016. Proteomics and comparative genomics of *Nitrososphaera viennensis* reveal the core genome and adaptations of archaeal ammonia oxidizers. Proc. Natl. Acad. Sci. 113, E7937–E7946. https://doi.org/10.1073/pnas.1601212113
Kieft, T.L., soroker, E., firestone, M.K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol. Biochem. 19, 119–126. https://doi.org/10.1016/0038-0717(87)90070-8
Klappenbach, J.A., Dunbar, J.M., Schmidt, T.M., 2000. rRNA operon copy number reflects ecological strategies of bacteria. Appl. Environ. Microbiol. 66, 1328–1333. https://doi.org/10.1128/AEM.66.4.1328-1333.2000
Kohout, P., Sýkorová, Z., Čtvrtlíková, M., Rydlová, J., Suda, J., Vohník, M., Sudová, R., 2012. Surprising spectra of root-associated fungi in submerged aquatic plants. FEMS Microbiol. Ecol. 80, 216–235. https://doi.org/10.1111/j.1574-6941.2011.01291.x
Konopka, A., Oliver, L., Turco, R.F., 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. Microb. Ecol. 35, 103–115. https://doi.org/10.1007/s002489900065
Koschorreck, M., 2000. Methane turnover in exposed sediments of an Amazon floodplain lake. Biogeochemistry 50, 195–206. https://doi.org/10.1023/A:1006326018597
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/AEM.01043-13
Lake, P.S., 2003. Ecological effects of perturbation by drought in flowing waters. Freshw. Biol. 48, 1161–1172. https://doi.org/10.1046/j.1365-2427.2003.01086.x
Lake, P.S., 2000. Disturbance, patchiness, and diversity in streams. J. North Am. Benthol. Soc. 19, 573–592. https://doi.org/10.2307/1468118
Langenheder, S., Lindström, E.S., 2019. Factors influencing aquatic and terrestrial bacterial community assembly. Environ. Microbiol. Rep. https://doi.org/10.1111/1758-2229.12731
Larned, S.T., Datry, T., Arscott, D.B., Tockner, K., 2010. Emerging concepts in temporary-river ecology. Freshw. Biol. 55, 717–738. https://doi.org/10.1111/j.1365-2427.2009.02322.x
Lear, G., Bellamy, J., Case, B.S., Lee, J.E., Buckley, H.L., 2014. Fine-scale spatial patterns in bacterial community composition and function within freshwater ponds. ISME J. 8, 1715–1726. https://doi.org/10.1038/ismej.2014.21
Ledger, M.E., Harris, R.M.L., Armitage, P.D., Milner, A.M., 2012. Climate Change Impacts on Community Resilience. Evidence from a Drought Disturbance Experiment, 1st ed, Advances in Ecological Research. Elsevier Ltd. https://doi.org/10.1016/B978-0-12-396992-7.00003-4
Legendre, P., Legendre, L., 1998. Numerical ecology. Numer. Ecol. Second English Ed. 20, 870. https://doi.org/10.1021/ic050220j
Lesk, C., Rowhani, P., Ramankutty, N., 2016. Influence of extreme weather disasters on global crop production. Nature 529, 84–87. https://doi.org/10.1038/nature16467

Levine, U.Y., Teal, T.K., Robertson, G.P., Schmidt, T.M., 2011. Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. ISME J. 5, 1683–1691. https://doi.org/10.1038/ismej.2011.40
Li, L., Xu, M., Ali, M.E., Zhang, W., Duan, Y., Li, D., 2018. Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. PLoS One 13, 1–18. https://doi.org/10.1371/journal.pone.0203812
Liu, W., Zhang, Z., Wan, S., 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob. Chang. Biol. 15, 184–195. https://doi.org/10.1111/j.1365-2486.2008.01728.x
Logue, J.B., Stedmon, C.A., Kellerman, A.M., Nielsen, N.J., Andersson, A.F., Laudon, H., Lindström, E.S., Kritzberg, E.S., 2016. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. ISME J. 10, 533–545. https://doi.org/10.1038/ismej.2015.131
Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: An effective distance metric for microbial community comparison. ISME J. 5, 169–172. https://doi.org/10.1038/ismej.2010.133
Malard, F., Tockner, K., Ward, J. V., 2000. Physico-chemical heterogeneity in a glacial riverscape. Landsc. Ecol. 15, 679–695. https://doi.org/10.1023/A:1008147419478
Manini, E., Danovaro, R., 2006. Synoptic determination of living/dead and active/dormant bacterial fractions in marine sediments. FEMS Microbiol. Ecol. 55, 416–423. https://doi.org/10.1111/j.1574-6941.2005.00042.x
Manzoni, S., Schaeffer, S.M., Katul, G., Porporato, A., Schimel, J.P., 2014. A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. Soil Biol. Biochem. 73. https://doi.org/10.1016/j.soilbio.2014.02.008
Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress : results from a meta-analysis 93, 930–938. https://doi.org/10.2307/23213741
Marcé, R., Obrador, B., Gómez-Gener, L., Catalán, N., Koschorreck, M., Arce, M.I., Singer, G., von Schiller, D., 2019. Emissions from dry inland waters are a blind spot in the global carbon cycle. Earth-Science Rev. 188, 240–248. https://doi.org/10.1016/j.earscirev.2018.11.012
Marmonier, P., Archambaud, G., Belaidi, N., Bougon, N., Breil, P., Chauvet, E., Claret, C., Cornut, J., Datry, T., Dole-Olivier, M.-J., Dumont, B., Flipo, N., Foulquier, A., Gérino, M., Guilpart, A., Julien, F., Maazouzi, C., Martin, D., Mermillod-Blondin, F., Montuelle, B., Namour, P., Navel, S., Ombredane, D., Pelte, T., Piscart, C., Pusch, M., Stroffek, S., Robertson, A., Sanchez-Pérez, J.-M., Sauvage, S., Taleb, A., Wantzen, M., Vervier, P., 2012. The role of organisms in hyporheic processes: gaps in current knowledge, needs for future research and applications. Ann. Limnol. - Int. J. Limnol. 48, 253–266. https://doi.org/10.1051/limn/2012009
Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. ISME J. 7, 830–838. https://doi.org/10.1038/ismej.2012.160
Marxsen, 2006. Bacterial production in the carbon flow of a central European stream, the Breitenbach. Freshwater Biology (2006) 51, 1838–1861. doi:10.1111/j.1365-2427.2006.01620.x
Marxsen, J., Zoppini, A., Wilczek, S., 2010. Microbial communities in streambed sediments recovering from desiccation. FEMS Microbiol. Ecol. 71, 374–386. https://doi.org/10.1111/j.1574-6941.2009.00819.x
Matz, C., Kjelleberg, S., 2005. Off the hook - How bacteria survive protozoan grazing. Trends Microbiol. 13, 302–307. https://doi.org/10.1016/j.tim.2005.05.009
Matz, C., McDougald, D., Moreno, A.M., Yung, P.Y., Yildiz, F.H., Kjelleberg, S., 2005. Biofilm formation and phenotypic variation enhance predation-driven persistence of Vibrio cholerae. Proc. Natl. Acad. Sci. 102, 16819–16824. https://doi.org/10.1073/pnas.0505350102
McDonough, O.T., Hosen, J.D., Palmer, M. a, 2011. Temporary streams: The hydrology, geography, and ecology of non-perennially flowing waters, River Ecosystems: Dynamics, Management and Conservation.
Mcknight, D.M., Niyogi, D.K., Alger, A.S., Bomblies, A., Conovitz, A., Tate, M., 1999. Dry Valley Streams in Antartica: Ecosystems Waiting for Water. Bioscience 49, 985–995. https://doi.org/https://doi.org/10.1525/bisi.1999.49.12.985
Mclaughlin, B.C., Ackerly, D.D., Klos, P.Z., Natali, J., Dawson, T.E., Thompson, S.E., 2017. Hydrologic refugia, plants, and climate change. Glob. Chang. Biol. 23, 2941–2961. https://doi.org/10.1111/gcb.13629
McLaughlin, C., 2008. Evaporation as a nutrient retention mechanism at Sycamore Creek, Arizona. Hydrobiologia 603, 241–252. https://doi.org/10.1007/s10750-007-9275-y
Medeiros, A.O., Pascoal, C., Graça, M.A.S., 2009. Diversity and activity of aquatic fungi under low oxygen conditions. Freshw. Biol. 54, 142–149. https://doi.org/10.1111/j.1365-2427.2008.02101.x
Medlin, L., Elwood, H.J., Stickel, S., Sogin, M.L., 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499. https://doi.org/10.1016/0378-1119(88)90066-2
Meisner, A., Jacquiod, S., Snoek, B.L., Ten Hooven, F.C., van der Putten, W.H., 2018. Drought legacy effects on the composition of soil fungal and prokaryote communities. Front. Microbiol. 9, 1–12. https://doi.org/10.3389/fmicb.2018.00294
Meisner, A., Leizeaga, A., Rousk, J., Bååth, E., 2017. Partial drying accelerates bacterial growth recovery to rewetting. Soil Biol. Biochem. 112, 269–276. https://doi.org/10.1016/j.soilbio.2017.05.016
Meisner, A., Rousk, J., Bååth, E., 2015. Prolonged drought changes the bacterial growth response to rewetting. Soil Biol. Biochem. 88, 314–322. https://doi.org/10.1016/j.soilbio.2015.06.002

Merbt, S.N., Proia, L., Prosser, J.I., Marti, E., Casamayor, E.O., Von Schiller, D., 2016. Stream drying drives microbial ammonia oxidation and first-flush nitrate export. Ecology 97, 2192–2198. https://doi.org/10.1002/ecy.1486
Mermillod-Blondin, F., Mauclaire, L., Montuelle, B., 2005. Use of slow filtration columns to assess oxygen respiration, consumption of dissolved organic carbon, nitrogen transformations, and microbial parameters in hyporheic sediments. Water Res. 39, 1687–1698. https://doi.org/10.1016/j.watres.2005.02.003
Milano, M., Ruelland, D., Fernandez, S., Dezetter, A., Fabre, J., Servat, E., Fritsch, J.M., Ardoin-Bardin, S., Thivet, G., 2013. Current state of Mediterranean water resources and future trends under climatic and anthropogenic changes. Hydrol. Sci. J. 58, 498–518. https://doi.org/10.1080/02626667.2013.774458
Mille-Lindblom, C., Von Wachenfeldt, E., Tranvik, L.J., 2004. Ergosterol as a measure of living fungal biomass: Persistence in environmental samples after fungal death. J. Microbiol. Methods 59, 253–262. https://doi.org/10.1016/j.mimet.2004.07.010
Monard, C., Gantner, S., Bertilsson, S., Hallin, S., Stenlid, J., 2016. Habitat generalists and specialists in microbial communities across a terrestrial-freshwater gradient. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep37719
Monroy, S., Menéndez, M., Basaguren, A., Pérez, J., Elosegi, A., Pozo, J., 2016. Drought and detritivores determine leaf litter decomposition in calcareous streams of the Ebro catchment (Spain). Sci. Total Environ. 573, 1450–1459. https://doi.org/10.1016/j.scitotenv.2016.07.209
Montgomery, H.J., Monreal, C.M., Young, J.C., Seifert, K.A., 2000. Determination of Soil Fungal Biomass from Soil Ergosterol Analyses. Soil Biol. Biochem. 32, 1207–1217.
Mora-Gómez, J., Duarte, S., Cássio, F., Pascoal, C., Romaní, A.M., 2018. Microbial decomposition is highly sensitive to leaf litter emersion in a permanent temperate stream. Sci. Total Environ. 621, 486–496. https://doi.org/10.1016/j.scitotenv.2017.11.055
Mora-Gómez, J., Elosegi, A., Duarte, S., Cássio, F., Pascoal, C., Romaní, A.M., Mora-Gomez, J., Elosegi, A., Duarte, S., Cássio, F., Pascoal, C., Romaní, A.M., 2016. Differences in the sensitivity of fungi and bacteria to season and invertebrates affect leaf litter decomposition in a Mediterranean stream. FEMS Microbiol. Ecol. 92, 1–35. https://doi.org/10.1093/femsec/fiw121
Mora-Gómez, J., Elosegi, A., Mas-Martí, E., Romaní, A.M., 2015. Factors controlling seasonality in leaf-litter breakdown in a Mediterranean stream. Freshw. Sci. 34, 1245–1258. https://doi.org/10.1086/683120
Morandi, B., Piégay, H., Lamouroux, N., Vaudor, L., 2014. How is success or failure in river restoration projects evaluated? Feedback from French restoration projects. J. Environ. Manage. 137, 178–188. https://doi.org/10.1016/j.jenvman.2014.02.010
Mori, N., Simčič, T., Brancelj, A., Robinson, C.T., Doering, M., 2017. Spatiotemporal heterogeneity of actual and potential respiration in two contrasting floodplains. Hydrol. Process. 31, 2622–2636. https://doi.org/10.1002/hyp.11211
Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol. Biochem. 59, 72–85. https://doi.org/10.1016/j.soilbio.2013.01.002
Moyano, F.E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, A., Epron, D., Formanek, P., Franzluebbers, A., Ilstedt, U., Kätterer, T., Orchard, V., Reichstein, M., Rey, A., Ruamps, L., Subke, J.A., Thomsen, I.K., Chenu, C., 2012. The moisture response of soil heterotrophic respiration: Interaction with soil properties. Biogeosciences 9, 1173–1182. https://doi.org/10.5194/bg-9-1173-2012
Muńiz, S., Lacarta, J., Pata, M.P., Jiménez, J.J., Navarro, E., 2014. Analysis of the diversity of substrate utilisation of soil bacteria exposed to Cd and earthworm activity using generalised additive models. PLoS One 9, 1–10. https://doi.org/10.1371/journal.pone.0085057
Nabhan, G.P., 1979. The ecology of floodwater farming in arid southwestern North America. Agro-Ecosystems 5, 245–255. https://doi.org/10.1016/0304-3746(79)90004-0
Nakano, S., Murakami, M., 2002. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. Proc. Natl. Acad. Sci. 98, 166–170. https://doi.org/10.1073/pnas.98.1.166
Naylor, D., Coleman-Derr, D., 2018. Drought Stress and Root-Associated Bacterial Communities. Front. Plant Sci. 8, 1–16. https://doi.org/10.3389/fpls.2017.02223
Naylor, D.T., 2017. The role of drought on root-associated bacterial communities across diverse cereal grass species and over a developmental gradient. UC Berkeley.
Norf, H., Arndt, H., Weitere, M., 2007. Impact of local temperature increase on the early development of biofilm-associated ciliate communities. Oecologia 151, 341–350. https://doi.org/10.1007/s00442-006-0545-6
Norf, H., Weitere, M., 2010. Resource quantityand seasonal background alter warming effects on communities of biofilm ciliates. FEMS Microbiol. Ecol. 74, 361–370. https://doi.org/10.1111/j.1574-6941.2010.00948.x
Noy-Meir, 1973. Desert Ecosystems : Environment and Producers. Annu. Rev. Ecol. Syst. 4, 25–51.
Obrador, B., von Schiller, D., Marcé, R., Gómez-Gener, L., Koschorreck, M., Borrego, C., Catalán, N., 2018. Dry habitats sustain high CO2 emissions from temporary ponds across seasons. Sci. Rep. 8, 3015. https://doi.org/10.1038/s41598-018-20969-y
Or, D., Smets, B.F., Wraith, J.M., Dechesne, A., Friedman, S.P., 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review. Adv. Water Resour. 30, 1505–1527. https://doi.org/10.1016/j.advwatres.2006.05.025
Orchard, V. a., Cook, F.J., 1983. Relationship between soil respiration and soil moisture. Soil Biol. Biochem. 15, 447–

453. https://doi.org/10.1016/0038-0717(83)90010-X
Ozturk, T., Ceber, Z.P., Türkeş, M., Kurnaz, M.L., 2015. Projections of climate change in the Mediterranean Basin by using downscaled global climate model outputs. Int. J. Climatol. 35, 4276–4292. https://doi.org/10.1002/joc.4285
Perujo, N., Sanchez-Vila, X., Proia, L., Romaní, A.M., 2017. Interaction between Physical Heterogeneity and Microbial Processes in Subsurface Sediments: A Laboratory-Scale Column Experiment. Environ. Sci. Technol. 51, 6110–6119. https://doi.org/10.1021/acs.est.6b06506
Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proc. Natl. Acad. Sci. 109, 10931–10936. https://doi.org/10.1073/pnas.1204306109
Pohlon, E., Mätzig, C., Marxsen, J., 2013a. Desiccation affects bacterial community structure and function in temperate stream sediments. Fundam. Appl. Limnol. / Arch. für Hydrobiol. 182, 123–134. https://doi.org/10.1127/1863-9135/2013/0465
Pohlon, E., Ochoa Fandino, A., Marxsen, J., 2013b. Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting. PLoS One 8, e83365. https://doi.org/10.1371/journal.pone.0083365
Poquet, J.M., Alba-Tercedor, J., Puntí, T., Del Mar Sánchez-Montoya, M., Robles, S., Álvarez, M., Zamora-Muñoz, C., Sáinz-Cantero, C.E., Vidal-Abarca, M.R., Suárez, M.L., Toro, M., Pujante, A.M., Rieradevall, M., Prat, N., 2009. The MEDiterranean Prediction and Classification System (MEDPACS): An implementation of the RIVPACS/AUSRIVAS predictive approach for assessing Mediterranean aquatic macroinvertebrate communities. Hydrobiologia 623, 153–171. https://doi.org/10.1007/s10750-008-9655-y
Portillo, M.C., Anderson, S.P., Fierer, N., 2012. Temporal variability in the diversity and composition of stream bacterioplankton communities. Environ. Microbiol. 14, 2417–2428. https://doi.org/10.1111/j.1462-2920.2012.02785.x
Potts, M., 1994. Desiccation tolerance of prokaryotes. Microbiol. Rev. 58, 755–805. https://doi.org/10.1093/icb/45.5.800
Pringle, C., 2003. What is hydrologic connectivity and why is it ecologically important? Hydrol. Process. 17, 2685–2689. https://doi.org/10.1002/hyp.5145
Prudhomme, C., Giuntoli, I., Robinson, E.L., Clark, D.B., Arnell, N.W., Dankers, R., Fekete, B.M., Franssen, W., Gerten, D., Gosling, S.N., Hagemann, S., Hannah, D.M., Kim, H., Masaki, Y., Satoh, Y., Stacke, T., Wada, Y., Wisser, D., 2014. Hydrological droughts in the 21st century, hotspots and uncertainties from a global multimodel ensemble experiment. Proc. Natl. Acad. Sci. 111, 3262–3267. https://doi.org/10.1073/pnas.1222473110
Pusch, M., Fiebig, I., Brettar, H., Eisenmann, H., Ellis, B.K., Kaplan, L.A., Lock, M.A., Naegeli, M.W., Traunspurger, W., 1998. The role of micro-organisms in the ecological connectivity of running waters. Freshw. Biol. 40, 453–495. https://doi.org/10.1046/j.1365-2427.1998.00372.x
Raymond, P.A., Hartmann, J., Lauerwald, R., Sobek, S., McDonald, C., Hoover, M., Butman, D., Striegl, R., Mayorga, E., Humborg, C., Kortelainen, P., Dürr, H., Meybeck, M., Ciais, P., Guth, P., 2013. Global carbon dioxide emissions from inland waters. Nature2013 503, 355. https://doi.org/10.1038/nature12760
Rees, G.N., Watson, G.O., Baldwin, D.S., Mitchell, A.M., 2006. Variability in sediment microbial communities in a semipermanent stream: impact of drought. J. North Am. Benthol. Soc. 25, 370–378. https://doi.org/10.1899/0887-3593(2006)25[370:vismci]2.0.co;2
Reynolds, J.F., Kemp, P.R., Ogle, K., Fernández, R.J., 2004. Modifying the “pulse-reserve” paradigm for deserts of North America: Precipitation pulses, soil water, and plant responses. Oecologia 141, 194–210. https://doi.org/10.1007/s00442-004-1524-4
Risse-Buhl, U., Felsmann, K., Mutz, M., 2014. Colonization dynamics of ciliate morphotypes modified by shifting sandy sediments. Eur. J. Protistol. 50, 345–355. https://doi.org/10.1016/j.ejop.2014.03.006
Risse-Buhl, U., Karsubke, M., Schlief, J., Baschien, C., Weitere, M., Mutz, M., 2012. Aquatic protists modulate the microbial activity associated with mineral surfaces and leaf litter. Aquat. Microb. Ecol. 66, 133–147. https://doi.org/10.3354/ame01564
Roberson, E.B., Firestone, M.K., 1992. Relationship between Desiccation and Exopolysaccharide Production in a Soil Pseudomonas sp . Appl. Environ. Microbiol. 58, 1284–1291. https://doi.org/PMC195588
Romaní, A.M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., Ylla, I., Zoppini, A., Romaní, A.M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., Ylla, I., Zoppini, A., Roman??, A.M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., Ylla, I., Zoppini, A., 2013. Microbial biofilm structure and organic matter use in mediterranean streams. Hydrobiologia 719, 43–58. https://doi.org/10.1007/s10750-012-1302-y
Romaní, A.M., Artigas, J., Ylla, I., 2012. Extracellular Enzymes in Aquatic Biofilms: Microbial Interactions versus Water Quality Effects in the Use of Organic Matter, in: Lear, G., Lewis, G.D. (Eds.), Microbial Biofilms: Current Research and Applications. Caister Academic Press, U.K.
Romaní, A.M., Borrego, C.M., Díaz-Villanueva, V., Freixa, A., Gich, F., Ylla, I., 2014. Shifts in microbial community structure and function in light- and dark-grown biofilms driven by warming. Environ. Microbiol. 16, 2550–2567. https://doi.org/10.1111/1462-2920.12428
Romaní, A.M., Chauvet, E., Febria, C., Mora-Gómez, J., Risse-Buhl, U., Timoner, X., Weitere, M., Zeglin, L., 2017. The biota of intermittent rivers and ephemeral streams: prokaryotes, fungi and protozoans, in: Intermittent

Rivers and Ephemeral Streams - Ecology and Management. Elsevier Inc. All rights reserved.
Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006a. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559–2569. https://doi.org/10.1890/0012-9658(2006)87[2559:IOBAFO]2.0.CO;2
Romaní, A.M., Fischer, H., Mille-lindblom, C., Tranvik, L.J., Roman, A.M., Fischer, H., Mille-lindblom, C., Tranvik, L.J., 2016. Interactions of Bacteria and Fungi on Decomposing Litter : Differential Extracellular Enzyme Activities Stable URL : http://www.jstor.org/stable/20069266 INTERACTIONS OF BACTERIA AND FUNGI ON DECOMPOSING LITTER : DIFFERENTIAL EXTRACELLULAR ENZYME ACTIVITI 87, 2559–2569.
Romaní, A.M., Fund, K., Artigas, J., Schwartz, T., Sabater, S., Obst, U., 2008. Relevance of polymeric matrix enzymes during biofilm formation. Microb. Ecol. 56, 427–436. https://doi.org/10.1007/s00248-007-9361-8
Romaní, A.M., Sabater, S., 2001. Structure and Activity of Rock and Sand Biofilms in a Mediterranean Stream. Ecology 82, 3232–3245. https://doi.org/10.2307/2679846
Romaní, A.M., Vázquez, E., Butturini, A., 2006b. Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: Biogeochemical link across the stream-riparian interface. Microb. Ecol. 52, 501–512. https://doi.org/10.1007/s00248-006-9112-2
Ruiz-González, C., Niño-García, J.P., Berggren, M., Del Giorgio, P.A., 2018. Contrasting dynamics and environmental controls of dispersed bacteria along a hydrologic gradient. Adv. Oceanogr. Limnol. 8. https://doi.org/10.4081/aiol.2017.7232
Rulík, M., Spáčil, R., 2004. Extracellular enzyme activity within hyporheic sediments of a small lowland stream, in: Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2004.07.005
Sabater, S., Timoner, X., Borrego, C., Acuña, V., 2016. Stream biofilm responses to flow intermittency: from cells to ecosystems. Front. Environ. Sci. 4, 1–10. https://doi.org/10.3389/fenvs.2016.00014
Schewe, J., Hinke, J., Gerten, D., Haddeland, I., Arnell, N.W., Clark, D.B., Dankers, R., Eisner, S., 2014. Multimodel assessment of water scarcity under climate change. Proc. Natl. Acad. Sci. 111, 3245–3250. https://doi.org/10.1073/pnas.1222460110
Schimel, J., B.T.C. and W.M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394. https://doi.org/10.1890/06-0219
Schimel, J.P., Boot, C., Holden, P.A., Roux-Michollet, D., Parker, S., Schaeffer, S., Treseder, K.K., 2010. Enzyme activity and adaptation in dry soil. 19th World Congr. Soil Sci. Soil Solut. a Chang. World 17–20.
Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 1–11. https://doi.org/10.3389/fmicb.2012.00348
Schlief, J., Mutz, M., 2011. Leaf decay processes during and after a supra-seasonal hydrological drought in a temperate lowland stream. Int. Rev. Hydrobiol. 96, 633–655. https://doi.org/10.1002/iroh.201111322
Schmidt, P.A., Schmitt, I., Otte, J., Bandow, C., Römbke, J., Bálint, M., Rolshausen, G., 2018. Season-Long Experimental Drought Alters Fungal Community Composition but Not Diversity in a Grassland Soil. Microb. Ecol. 75, 468–478. https://doi.org/10.1007/s00248-017-1047-2
Seiler, C., van Velzen, E., Neu, T.R., Gaedke, U., Berendonk, T.U., Weitere, M., 2017. Grazing resistance of bacterial biofilms: A matter of predators' feeding trait. FEMS Microbiol. Ecol. 93, 1–9. https://doi.org/10.1093/femsec/fix112
Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M., Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. Front. Microbiol. 3, 1–19. https://doi.org/10.3389/fmicb.2012.00417
Sheldon, F., Bunn, S.E., Hughes, J.M., Arthington, A.H., Balcombe, S.R., Fellows, C.S., 2010. Ecological roles and threats to aquatic refugia in arid landscapes: dryland river waterholes. Mar. Freshw. Res. 61, 885. https://doi.org/10.1071/mf09239
Shentsis, I., Rosenthal, E., 2003. Recharge of aquifers by flood events in an arid region. Hydrol. Process. 17, 695–712. https://doi.org/10.1002/hyp.1160
Shumilova, O., Zak, D., Datry, T., von Schiller, D., Corti, R., Foulquier, A., Obrador, B., Tockner, K., Allan, D.C., Altermatt, F., Arce, M.I., Arnon, S., Banas, D., Banegas-Medina, A., Beller, E., Blanchette, M.L., Blanco-Libreros, J.F., Blessing, J., Boëchat, I.G., Boersma, K., Bogan, M.T., Bonada, N., Bond, N.R., Brintrup, K., Bruder, A., Burrows, R., Cancellario, T., Carlson, S.M., Cauvy-Fraunié, S., Cid, N., Danger, M., de Freitas Terra, B., Girolamo, A.M. De, Campo, R. del, Dyer, F., Elosegi, A., Faye, E., Febria, C., Figueroa, R., Four, B., Gessner, M.O., Gnohossou, P., Cerezo, R.G., Gomez-Gener, L., Graça, M.A.S., Guareschi, S., Gücker, B., Hwan, J.L., Kubheka, S., Langhans, S.D., Leigh, C., Little, C.J., Lorenz, S., Marshall, J., McIntosh, A., Mendoza-Lera, C., Meyer, E.I., Miliša, M., Mlambo, M.C., Moleón, M., Negus, P., Niyogi, D., Papatheodoulou, A., Pardo, I., Paril, P., Pešić, V., Rodriguez-Lozano, P., Rolls, R.J., Sanchez-Montoya, M.M., Savić, A., Steward, A., Stubbington, R., Taleb, A., Vorste, R. Vander, Waltham, N., Zoppini, A., Zarfl, C., 2019. Simulating rewetting events in intermittent rivers and ephemeral streams: A global analysis of leached nutrients and organic matter. Glob. Chang. Biol. 1591–1611. https://doi.org/10.1111/gcb.14537
Simon, M., López-García, P., Deschamps, P., Restoux, G., Bertolino, P., Moreira, D., Jardillier, L., 2016. Resilience of freshwater communities of small microbial eukaryotes undergoing severe drought events. Front. Microbiol. 7, 1–11. https://doi.org/10.3389/fmicb.2016.00812
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–

404. https://doi.org/10.1016/j.soilbio.2009.10.014
Skoulikidis, N.T., Sabater, S., Datry, T., Morais, M.M., Buffagni, A., Dörflinger, G., Zogaris, S., del Mar Sánchez-Montoya, M., Bonada, N., Kalogianni, E., Rosado, J., Vardakas, L., De Girolamo, A.M., Tockner, K., 2017. Non-perennial Mediterranean rivers in Europe: Status, pressures, and challenges for research and management. Sci. Total Environ. 577, 1–18. https://doi.org/10.1016/j.scitotenv.2016.10.147
Snelder, T.H., Datry, T., Lamouroux, N., Larned, S.T., Sauquet, E., Pella, H., Catalogne, C., 2013. Regionalization of patterns of flow intermittence from gauging station records. Hydrol. Earth Syst. Sci. 17, 2685–2699. https://doi.org/10.5194/hess-17-2685-2013
Spang, A., Saw, J.H., Jørgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., Van Eijk, R., Schleper, C., Guy, L., Ettema, T.J.G., 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521, 173–179. https://doi.org/10.1038/nature14447
Stahl, D.A., de la Torre, J.R., 2012. Physiology and Diversity of Ammonia-Oxidizing Archaea. Annu. Rev. Microbiol. 66, 83–101. https://doi.org/10.1146/annurev-micro-092611-150128
Stanford, J.A., Lorang, M.S., Hauer, F.R., 2005. The shifting habitat mosaic of river ecosystems. SIL Proceedings, 1922-2010 29, 123–136. https://doi.org/10.1080/03680770.2005.11901979
Stanley, E.H., Fisher, S.G., Grimm, N.B., 1997. Ecosystem Expansion and Contraction in Streams. Bioscience 47, 427–435. https://doi.org/10.2307/1313058
Stefan, G., Cornelia, B., Römbke, J., Bonkowski, M., 2014. Soil water availability strongly alters the community composition of soil protists. Pedobiologia (Jena). 57, 205–213. https://doi.org/10.1016/j.pedobi.2014.10.001
Stegen, J.C., Fredrickson, J.K., Wilkins, M.J., Konopka, A.E., Nelson, W.C., Arntzen, E. V., Chrisler, W.B., Chu, R.K., Danczak, R.E., Fansler, S.J., Kennedy, D.W., Resch, C.T., Tfaily, M., 2016. Groundwater-surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover. Nat. Commun. 7, 11237. https://doi.org/10.1038/ncomms11237
Steven, B., Hesse, C., Soghigian, J., Gallegos-Graves, L.V., Dunbar, J., 2017. Simulated rRNA/DNA Ratios Show Potential To Misclassify Active Populations as Dormant. Appl. Environ. Microbiol. 83, 1–11. https://doi.org/10.1128/aem.00696-17
Stevenson, A., Hallsworth, J.E., 2014. Water and temperature relations of soil Actinobacteria. Environ. Microbiol. Rep. 6, 744–755. https://doi.org/10.1111/1758-2229.12199
Steward, A.L., 2012. When the river runs dry: the ecology of dry river beds. Griffith University.
Steward, A.L., Negus, P., Marshall, J.C., Clifford, S.E., Dent, C., 2018. Assessing the ecological health of rivers when they are dry. Ecol. Indic. 85, 537–547. https://doi.org/10.1016/j.ecolind.2017.10.053
Steward, A.L., Von Schiller, D., Tockner, K., Marshall, J.C., Bunn, S.E., 2012. When the river runs dry: Human and ecological values of dry riverbeds. Front. Ecol. Environ. 10, 202–209. https://doi.org/10.1890/110136
Stieglmeier, M., Klingl, A., Alves, R.J.E., Rittmann, S.K.M.R., Melcher, M., Leisch, N., Schleper, C., 2014. Nitrososphaera viennensis gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon from soil and a member of the archaeal phylum Thaumarchaeota. Int. J. Syst. Evol. Microbiol. 64, 2738–2752. https://doi.org/10.1099/ijs.0.063172-0
Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x
Storey, R.G., Fulthorpe, R.R., Williams, D.D., 1999. Perspectives and predictions on the microbial ecology of the hyporheic zone. Freshw. Biol. 41, 119–130. https://doi.org/10.1046/j.1365-2427.1999.00377.x
Stubbington, R., Chadd, R., Cid, N., Csabai, Z., Mili, M., Morais, M., Munné, A., Pa, P., Pe, V., Tziortzis, I., Verdonschot, R.C.M., Datry, T., 2017. Biomonitoring of intermittent rivers and ephemeral streams in Europe : Current practice and priorities to enhance ecological status assessments. https://doi.org/10.1016/j.scitotenv.2017.09.137
Suberkropp, K., Arsuffi, T.L., Anderson, J.P., 1983. Comparison of Degradative Ability , Enzymatic Activity , and Palatability of Aquatic Hyphomycetes Grown on Leaf Litter Comparison of Degradative Ability , Enzymatic Activity , and Palatability of Aquatic Hyphomycetes Grown on Leaf Litter. Appl. Environ. Microbiol. 46, 237–244. https://doi.org/0099-2240/83/070237-08$02.00/0
Sudheep, N.M., Sridhar, K.R., 2012. Aquatic hyphomycetes in hyporheic freshwater habitats of southwest India. Limnologica 42, 87–94. https://doi.org/10.1016/j.limno.2012.02.001
Székely, A.J., Langenheder, S., 2017. Dispersal timing and drought history influence the response of bacterioplankton to drying-rewetting stress. ISME J. 11, 1–13. https://doi.org/10.1038/ismej.2017.55
Takai, K., Horikoshi, K., 2000. Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes. Appl. Environ. Microbiol. 66, 5066–5072. https://doi.org/10.1128/AEM.66.11.5066-5072.2000.Updated
Tamames, J., Abellán, J.J., Pignatelli, M., Camacho, A., Moya, A., 2010. Environmental distribution of prokaryotic taxa. BMC Microbiol. 10. https://doi.org/10.1186/1471-2180-10-85
Thion, C., Prosser, J.I., 2014. Differential response of nonadapted ammonia-oxidising archaea and bacteria to drying-rewetting stress. FEMS Microbiol. Ecol. 90, 380–389. https://doi.org/10.1111/1574-6941.12395
Thorp, J.H., Thoms, M.C., Delong, M.D., 2006. The riverine ecosystem synthesis: Biocomplexity in river networks

across space and time. River Res. Appl. 22, 123–147. https://doi.org/10.1002/rra.901
Timoner, X., Acuña, V., Frampton, L., Pollard, P., Sabater, S., Bunn, S.E., 2014a. Biofilm functional responses to the rehydration of a dry intermittent stream. Hydrobiologia 727, 185–195. https://doi.org/10.1007/s10750-013-1802-4
Timoner, X., Acuña, V., Von Schiller, D., Sabater, S., 2012. Functional responses of stream biofilms to flow cessation, desiccation and rewetting. Freshw. Biol. 57, 1565–1578. https://doi.org/10.1111/j.1365-2427.2012.02818.x
Timoner, X., Borrego, C.M., Acuña, V., Sabater, S., 2014b. The dynamics of biofilm bacterial communities is driven by flow wax and wane in a temporary stream. Limnol. Oceanogr. 59, 2057–2067. https://doi.org/10.4319/lo.2014.59.6.2057
Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x
Tranvik, L.J., Langenheder, S., Lindström, E.S., 2005. Weak Coupling between Community Composition and Functioning of Aquatic Bacteria. Limnol. Oceanogr. 50, 957–967.
Tzoraki, O., Nikolaidis, N.P., Amaxidis, Y., Skoulikidis, N.T., 2007. In-stream biogeochemical processes of a temporary river. Environ. Sci. Technol. 41, 1225–1231. https://doi.org/10.1021/es062193h
Unger, I.M., Kennedy, A.C., Muzika, R.-M., 2009. Flooding effects on soil microbial communities. Appl. Soil Ecol. 42, 1–8. https://doi.org/10.1016/j.apsoil.2009.01.007
Vadher, A.N., Leigh, C., Millett, J., Stubbington, R., Wood, P.J., 2017. Vertical movements through subsurface stream sediments by benthic macroinvertebrates during experimental drying are influenced by sediment characteristics and species traits. Freshw. Biol. 62, 1730–1740. https://doi.org/10.1111/fwb.12983
von Schiller, D., Acuña, V., Graeber, D., Martí, E., Ribot, M., Sabater, S., Timoner, X., Tockner, K., 2011. Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. Aquat. Sci. 73, 485–497. https://doi.org/10.1007/s00027-011-0195-6
von Schiller, D., Marcé, R., Obrador, B., Gómez-Gener, L., Casas-Ruiz, J., Acuña, V., Koschorreck, M., 2014. Carbon dioxide emissions from dry watercourses. Inl. Waters 4, 377–382. https://doi.org/10.5268/IW-4.4.746
Vorösmarty Pamela; ; Salisbury, Joseph; ; Lammers, Richard B., C.J.. ; G., 2000. Global Water Resources: Vulnerability from Climate Change and Population Growth. Science (80-.). 289, 284–288. https://doi.org/10.1126/science.289.5477.284
Wagner, K., Bengtsson, M.M., Besemer, K., Sieczko, A., Burns, N.R., Herberg, E.R., Battin, T.J., 2014. Functional and Structural Responses of Hyporheic Biofilms to Varying Sources of Dissolved Organic Matter. Appl. Environ. Microbiol. 80, 6004–6012. https://doi.org/10.1128/AEM.01128-14
Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109, 35–47. https://doi.org/10.1007/s10533-011-9641-8
Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S Ribosomal DNA Amplification for Phylogenetic Study. J. Bacteriol. 173, 697–703. https://doi.org/n.a.
Weitere, M., Erken, M., Majdi, N., Arndt, H., Norf, H., Reinshagen, M., Traunspurger, W., Walterscheid, A., Wey, J.K., 2018. The food web perspective on aquatic biofilms. Ecol. Monogr. 88, 543–559. https://doi.org/10.1002/ecm.1315
Westwood, C.G., Teeuw, R.M., Wade, P.M., Holmes, N.T.H., 2006. Prediction of macrophyte communities in drought-affected groundwater-fed headwater streams. Hydrol. Process. 20, 127–145. https://doi.org/10.1002/hyp.5907
Wey, J.K., Jürgens, K., Weiterea, M., 2012. Seasonal and successional influences on bacterial community composition exceed that of protozoan grazing in river biofilms. Appl. Environ. Microbiol. 78, 2013–2024. https://doi.org/10.1128/AEM.06517-11
Wilhite, D.A., 2000. Drought as a Natural Hazard: Concepts and Definitions, in: Drought: A Global Assessment. Drought Mitigation Center Faculty Publications, pp. 3–18.
Winemiller, K.O., Flecker, A.S., Hoeinghaus, D.J., 2010. Patch dynamics and environmental heterogeneity in lotic ecosystems. J. North Am. Benthol. Soc. 29, 84–99. https://doi.org/10.1899/08-048.1
Wood, J.M., Bremer, E., Csonka, L.N., Kraemer, R., Poolman, B., Van der Heide, T., Smith, L.T., 2001. Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 130, 437–460. https://doi.org/10.1016/S1095-6433(01)00442-1
Ylla, I., Borrego, C., Romaní, A.M., Sabater, S., 2009. Availability of glucose and light modulates the structure and function of a microbial biofilm: Research article. FEMS Microbiol. Ecol. 69, 27–42. https://doi.org/10.1111/j.1574-6941.2009.00689.x
Ylla, I., Romaní, A.M., Sabater, S., 2012. Labile and Recalcitrant Organic Matter Utilization by River Biofilm Under Increasing Water Temperature. Microb. Ecol. 64, 593–604. https://doi.org/10.1007/s00248-012-0062-6
Ylla, I., Sanpera-Calbet, I., Vázquez, E., Romaní, A.M., Muñoz, I., Butturini, A., Sabater, S., 2010. Organic matter availability during pre- and post-drought periods in a Mediterranean stream. Hydrobiologia 657, 217–232. https://doi.org/10.1007/s10750-010-0193-z
Yuste, J.C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., M., P., J., S., 2011. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob. Chang. Biol. 17, 1475–1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zeglin, L.H., 2015. Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Front. Microbiol. 6, 454. https://doi.org/10.3389/fmicb.2015.00454

Zhou, X., Fornara, D., Ikenaga, M., Akagi, I., Zhang, R., Jia, Z., 2016. The resilience of microbial community under drying and rewetting cycles of three forest soils. Front. Microbiol. 7, 1–12. https://doi.org/10.3389/fmicb.2016.01101

Zlatanović, S., Fabian, J., Premke, K., Mutz, M., 2017. Shading and sediment structure effects on stream metabolism resistance and resilience to infrequent droughts. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.10.105

Zoppini, A., Ademollo, N., Amalfitano, S., Capri, S., Casella, P., Fazi, S., Marxsen, J., Patrolecco, L., 2016. Microbial responses to polycyclic aromatic hydrocarbon contamination in temporary river sediments: Experimental insights. Sci. Total Environ. 541, 1364–1371. https://doi.org/10.1016/j.scitotenv.2015.09.144

Zoppini, A., Ademollo, N., Amalfitano, S., Casella, P., Patrolecco, L., Polesello, S., 2014. Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. Sci. Total Environ. 484, 74–83. https://doi.org/10.1016/j.scitotenv.2014.03.019

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2009. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1, 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

ANNEX (Supplementary materials)

CHAPTER 1:

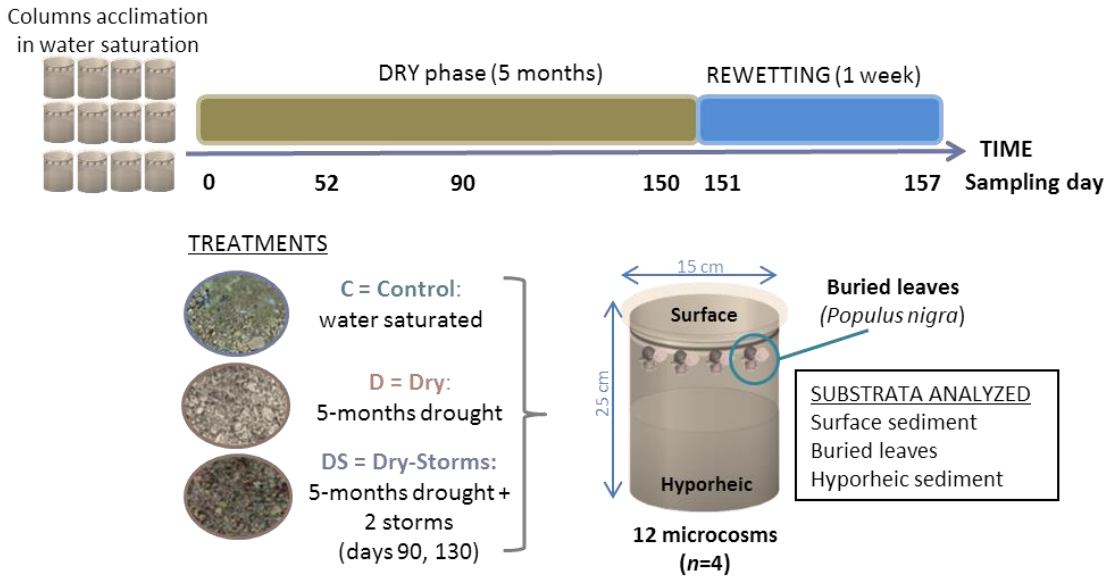


Fig. S1 The experimental design.

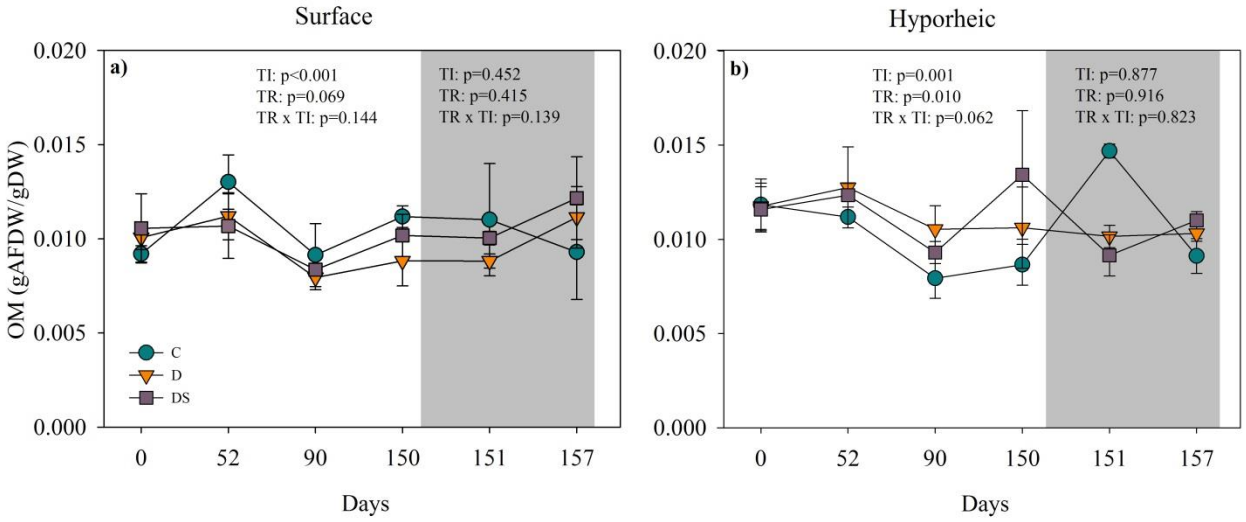


Figure S2. Organic matter content (gAFDW/gDW) for the three treatments (C: Control, D: Dry, DS: Dry-Storm) in surface and hyporheic sediments during the long-term drought and rewetting (shaded area) experimental phases. Values are mean ± SD (*n*=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

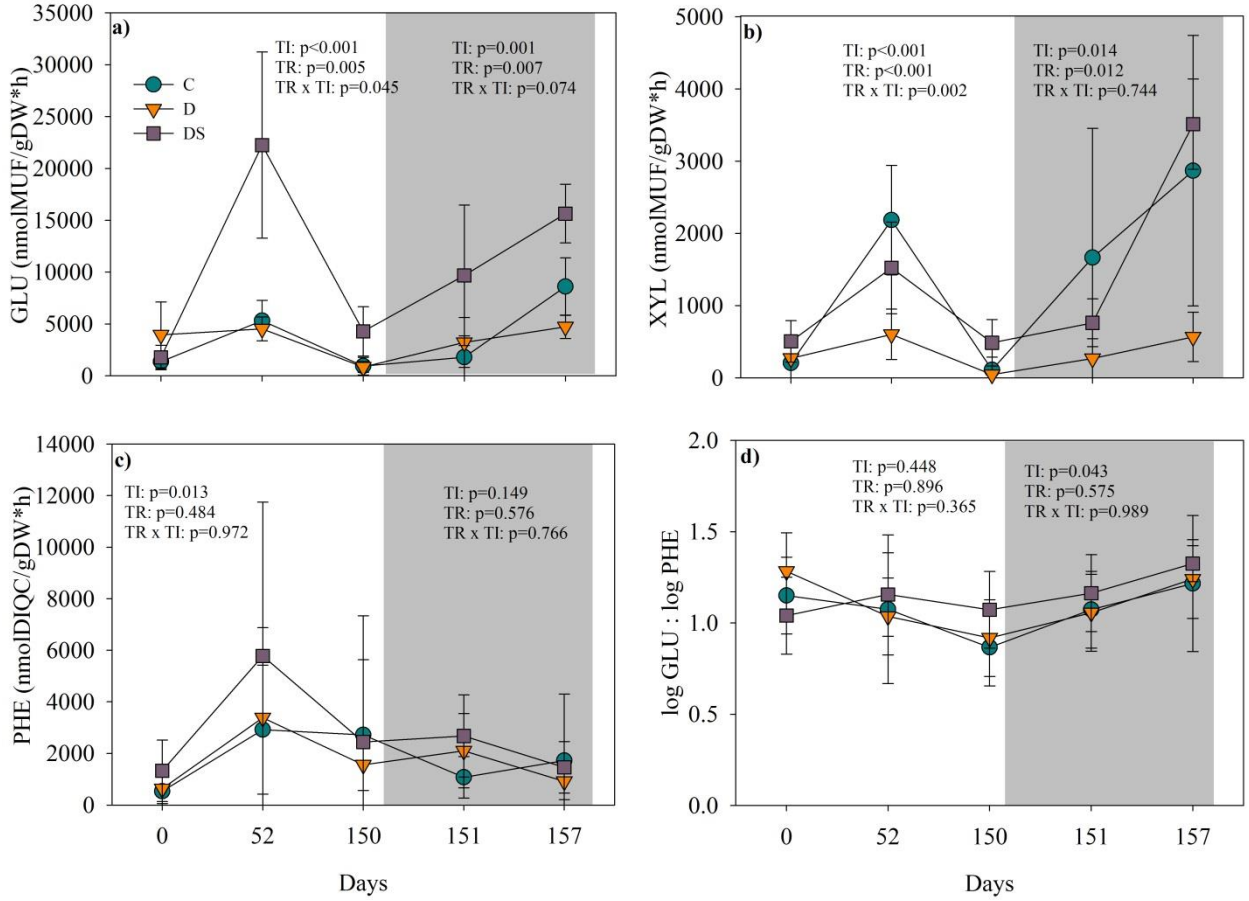


Figure S3. (a) β-D-glucosidase (GLU); (b) β-D-xylosidase (XYL); (c) phenol-oxidase (PHE), in leaf litter discs, during the long-term drought and rewetting (shaded area) experimental phases. (d) Recalcitrant index (GLU:PHE) is also shown. Values are mean ± SD (*n*=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

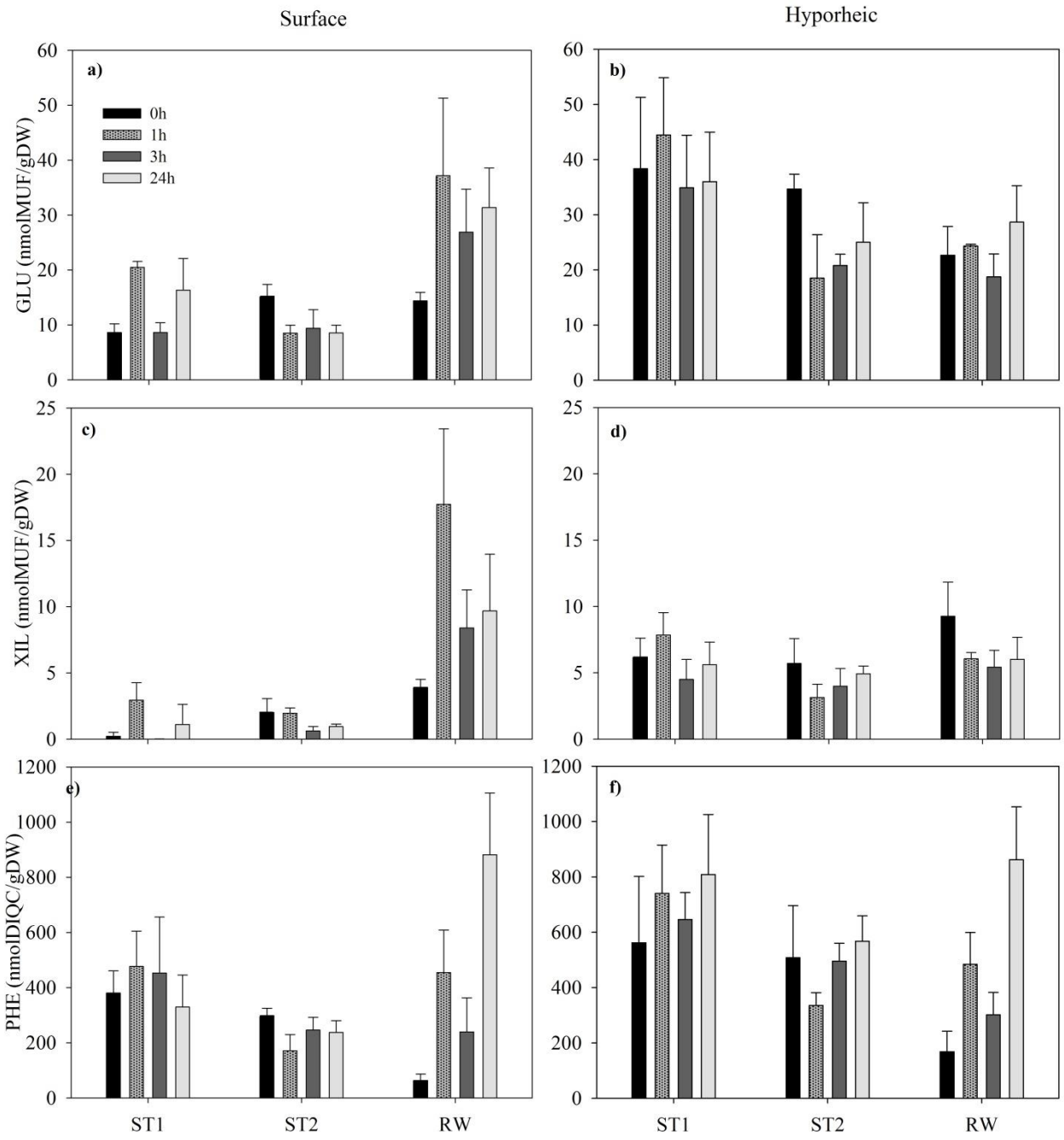


Figure S4. Extracellular enzymatic activities (GLU; XYL; PHE) immediately before (time 0h) and after 1, 3 and 24 hours from storm episodes (ST1: Storm 1; ST2: Storm 2) and rewetting (RW: Rewetting) in DS treatment from surface and hyporheic sediments. Values are mean ± SD (*n*=4).

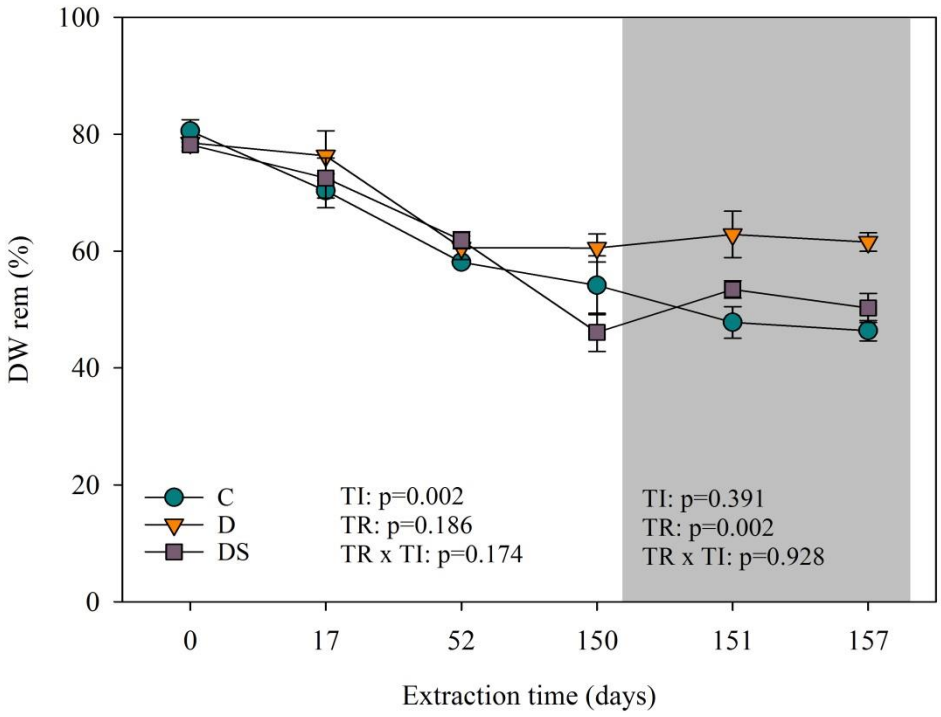


Figure S5. Percentage of dry weight remaining (%DW rem) in leaf litter during the long-term drought and rewetting (shaded area) experimental phases, for the three treatments (C: Control, D: Dry, DS: Dry-Storm). Values are mean ± SD (*n*=4). The p values referred to Linear Mixed Models, where TI and TR refer to time and treatment, respectively.

Table S1. ACA stream nutrients database in mg/L.

Database nutrients - ACA - Programa de Seguiment i Control -El Fluvià Riera de Bianya

Date	NH_4^+	PO_4^{3-}	NO_3^-	NO_2^-
2016/02/18	<0.2	<0.2	<5	<0.04
2016/02/18	<0.2	<0.2	8.5	<0.04
2016/02/18	<0.2	<0.2	9.9	<0.04
2016/02/29	0.2	0.7	14.4	<0.04
2016/02/29	<0.2	_	13.7	_
2016/02/29	0.2	_	11.1	_
2016/03/07	<0.2	0.8	_	_
2016/03/07	<0.2	_	_	_
2016/03/07	<0.2	_	_	<0.04
2016/04/12	<0.2	0.7	7.8	<0.04
2016/04/12	<0.2	<0.2	_	0.26
2016/04/12	<0.2	0.6	0.6	<0.04
2016/05/11	<0.2	0.6	<0.2	<0.04
2016/05/11	0.4	<0.2	1.1	<0.04
2016/05/11	0.5	1.1	<0.2	<0.04
2016/05/11	0.7	<0.2	<0.2	<0.04
2016/05/11	<0.2	<0.2	_	_
2016/05/11	<0.2	0.5	_	_
2016/06/30	0.2	0.4	0.5	_
2016/06/30	<0.2	2.3	_	_
2016/06/30	<0.2	0.9	_	<0.04
2016/07/14	0.3	<0.2	0.4	<0.04
2016/07/14	<0.2	<0.2	2.3	0.08
2016/07/14	<0.2	0.5	0.9	0.07

Table S2. Percentage of sediment particles with diameter <63 µm and <4 µm during the first 90 days of drying, before storms. Treatments are defined as C (Control), D (Dry) and DS (DryStorm). Mean indicates average values.

Surface						
	C		D		DS	
Dry day	%<63	%<4	%<63	%<4	%<63	%<4
0	8.12	2.26	6.79	1.45	7.12	1.93
52	7.71	2.32	3.79	0.91	6.24	1.8
90	4.76	1.11	3.59	0.88	4.13	0.99
Mean	6.86	1.90	4.72	1.08	5.83	1.57
Hyporheic						
	C		D		DS	
Dry day	%<63	%<4	%<63	%<4	%<63	%<4
0	9.46	2.36	8.00	2.38	8.82	2.51
52	7.2	1.37	7.92	1.68	7.74	1.64
90	3.86	0.98	4.04	1.04	3.21	0.82
Mean	6.84	1.57	6.66	1.70	6.59	1.66

Table S3. p-Values from linear mixed models (LMM). Fixed effects factors considered in the model: depth (DE), storm and final rewetting events (ST) and time (TI, repeated measures factor) during the experimental period (0, 1, 3, 24 hours after the incoming event).

Source of variation	GLU	XYL	PHE
	p value		
ST	**<0.001**	**<0.001**	**<0.001**
TI	**0.003**	**<0.001**	**<0.001**
DE	**<0.001**	**<0.001**	**<0.001**
ST x TI	**<0.001**	**0.002**	**<0.001**
ST x DE	**<0.001**	**<0.001**	0.137
TI x DE	**0.004**	**<0.001**	0.242
ST x TI x DE	**0.028**	**0.013**	**0.006**

Response variables: GLU, β-glucosidase; XYL, β-xylosidase; PHE, phenol oxidase. Significant p-values (<0.05) are indicated in boldface.

Table S4. p-Values from linear mixed models (LMM) for each depth compartment (surface and hyporheic sediment). Fixed effects factors considered in the model: storm and final rewetting events (ST) and time (TI, repeated measures factor) during the experimental period (0, 1, 3, 24 hours after the incoming event).

Source of variation	GLU	XYL	PHE
	p value		
Surface			
ST	**<0.001**	**<0.001**	**0.001**
TI	**<0.001**	**<0.001**	**<0.001**
ST x TI	**<0.001**	**0.002**	**<0.001**
Hyporheic			
ST	**<0.001**	**0.004**	**<0.001**
TI	0.083	**0.036**	**<0.001**
ST x TI	**0.023**	0.121	**<0.001**

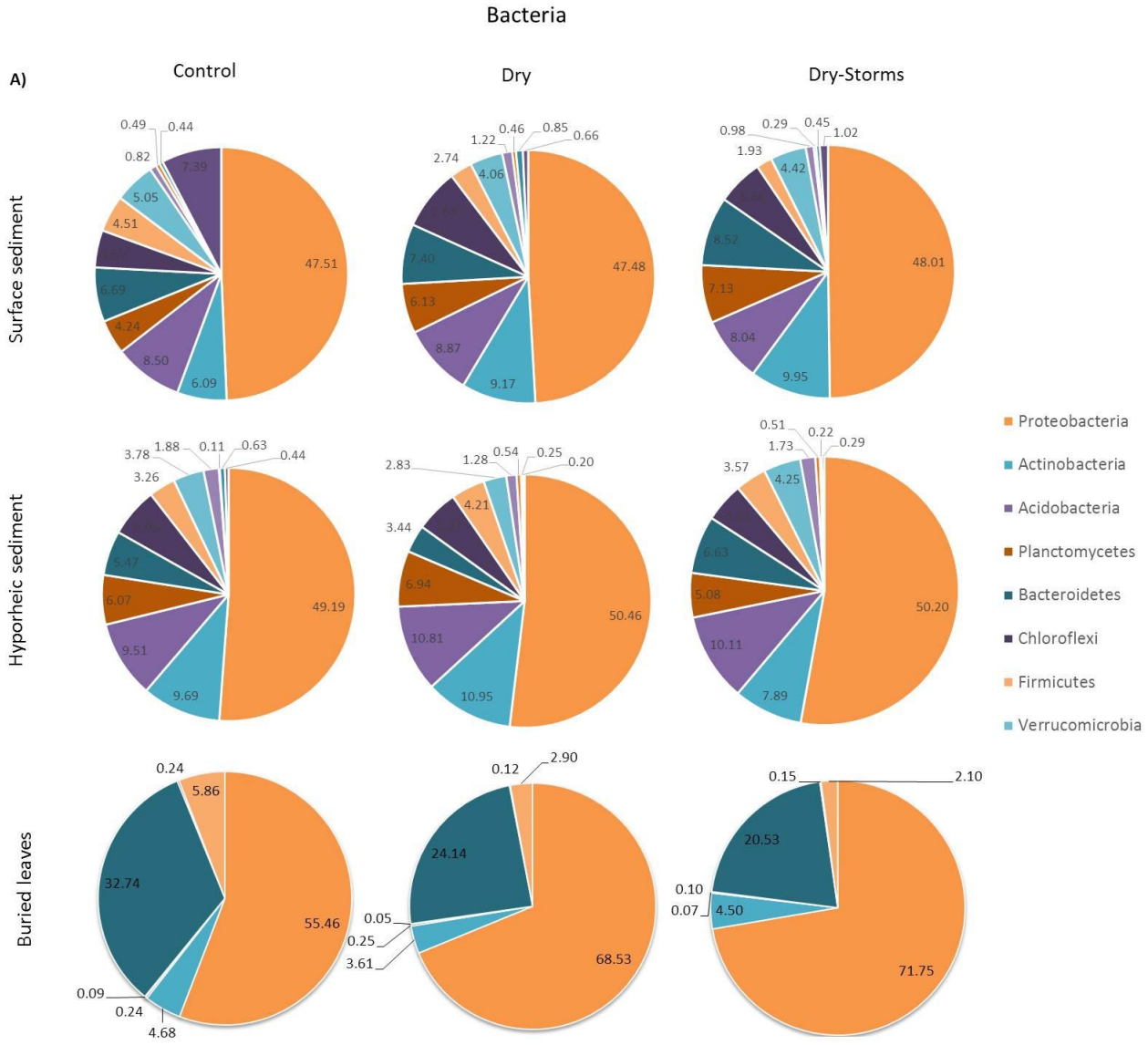
Response variables: GLU, β-glucosidase; XYL, β-xylosidase; PHE, phenol oxidase. Significant p-values (<0.05) are indicated in boldface.

Table S5. p-Values from one-way ANOVA results considering final rewetting values (day 157 or 165) of treatments (TR). When significant differences were found, final control values (C) were compared with final values from dry (D) and dry-storms (DS) treatments (pairwise comparisons with Dunnet's test).

Source of variation	Chl-a	Fungi	EPS	Bacteria	Live cells	GLU	XYL	PHE
Surface				*p values*				
TR	**0.003**	0.843	0.060	0.116	**0.007**	**0.011**	0.070	0.159
C X D	**<0.001**	0.784	0.154	0.808	0.337	**0.006**	0.119	0.163
C X DS	**<0.001**	0.951	**0.038**	0.207	**0.019**	0.116	0.861	0.188
Hyporheos								
TR	**0.029**	0.630	0.886	0.143	**0.033**	**<0.001**	**0.002**	**0.002**
C X D	0.128	0.557	0.991	0.104	0.998	**0.003**	**0.002**	0.261
C X DS	0.402	0.683	0.853	0.747	**0.041**	**<0.001**	**0.001**	**0.001**

Response variables: C: Control, D: Dry, DS: Dry-Storm treatment. Chl-a, chlorophyll-a; Fungi, fungal biomass; EPS, extracellular polymeric substances; Bacteria, bacterial biomass; Live cells, bacterial viability; GLU, β-glucosidase; XYL; β-xylosidase; PHE phenol oxidase. Significant p-values (<0.05) are indicated in boldface. EPS treatment result was close to significance for this reason we proceeded with pairwise comparisons.

CHAPTER 2


Bacteria
A)
Control
Dry
Dry-Storms
Surface sediment
Hyporheic sediment
Buried leaves
0.49
0.44
0.82
7.39
5.05
4.51
6.69
4.24
8.50
6.09
47.51
1.22
0.46
0.85
0.66
2.74
4.06
7.40
6.13
8.87
9.17
47.48
0.98
0.29
0.45
1.02
1.93
4.42
8.52
7.13
8.04
9.95
48.01
3.78
1.88
0.11
0.63
0.44
3.26
5.47
6.07
9.51
9.69
49.19
2.83
1.28
0.54
0.25
0.20
4.21
3.44
6.94
10.81
10.95
50.46
0.51
0.22
1.73
0.29
3.57
4.25
6.63
5.08
10.11
7.89
50.20
0.24
5.86
32.74
55.46
0.09
0.24
4.68
0.12
2.90
24.14
0.05
0.25
3.61
68.53
0.15
2.10
20.53
0.10
0.07
4.50
71.75
Proteobacteria
Actinobacteria
Acidobacteria
Planctomycetes
Bacteroidetes
Chloroflexi
Firmicutes
Verrucomicrobia

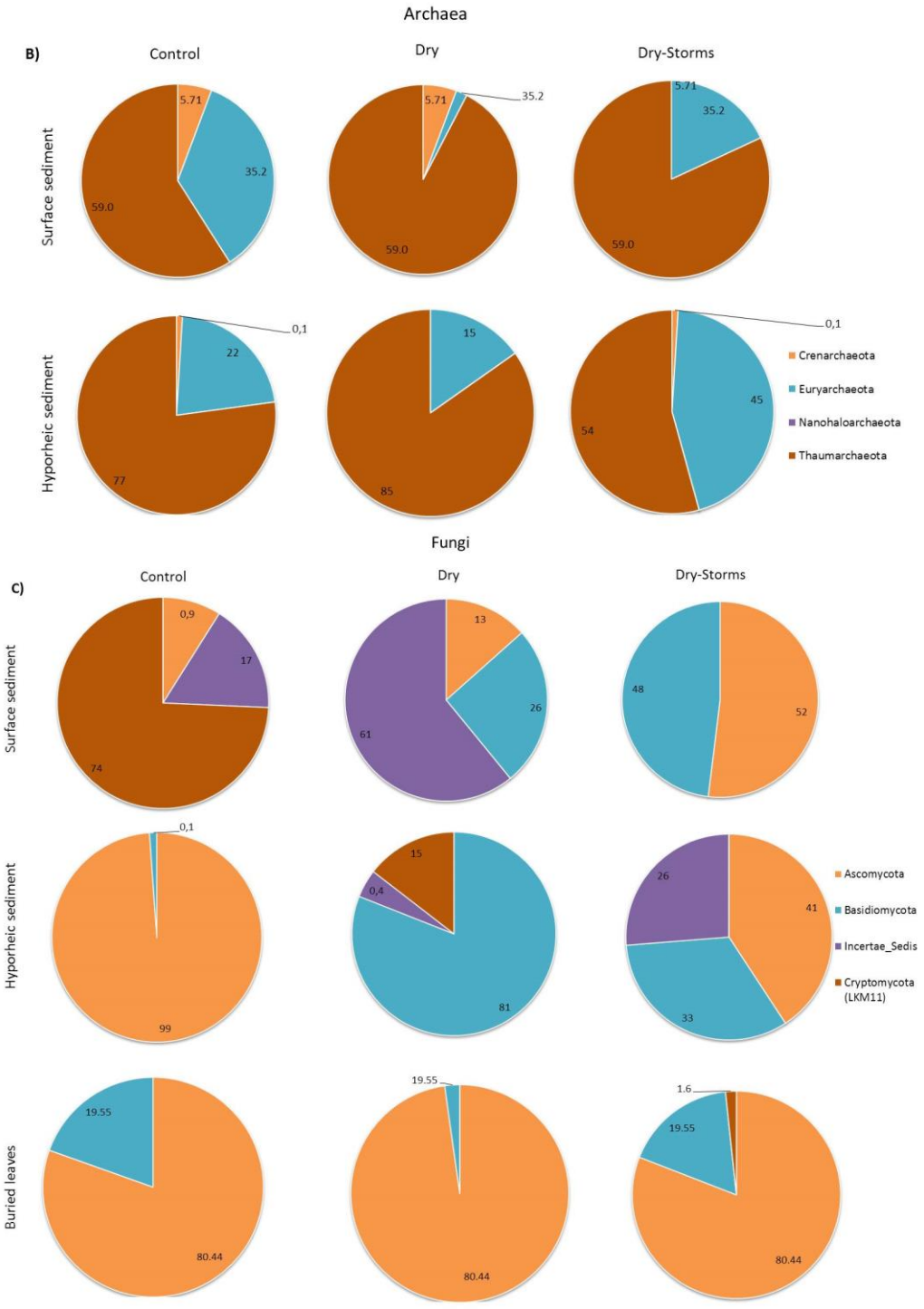


Fig. S1 Cake plots representing initial microbial composition (time 0) for Control, Dry and Dry-Storm treatments in surface and hyporheic sediments and buried leaves for: **A)** bacteria, **B)** archaea, **C)** fungi. These results are associated to those presented in the Table S1.

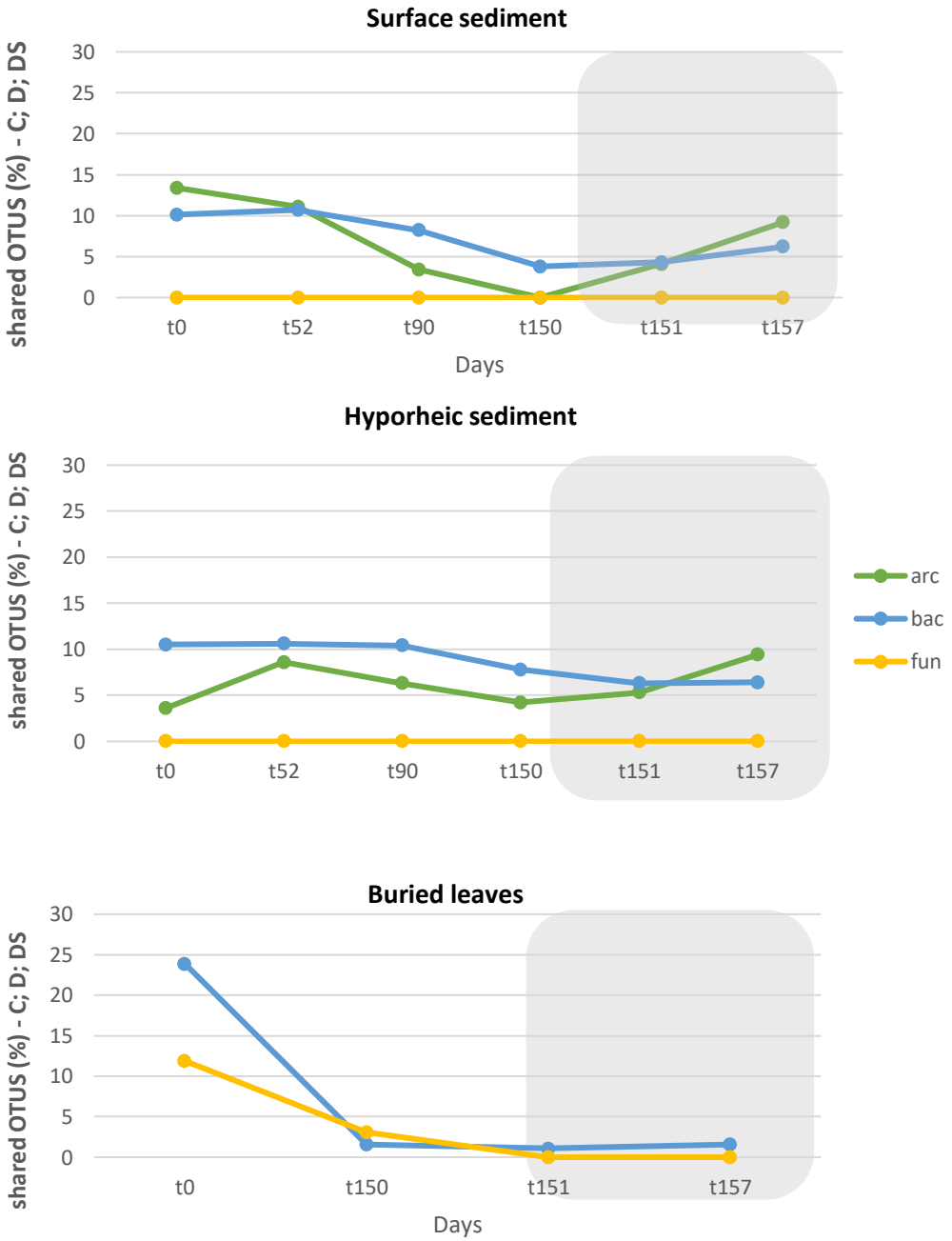


Fig. S2 Percentage of shared OTUs between the three treatments (C, Control; D, Dry; DS, Dry-Storm) reported during the experimental time (0, 52, 90, 150, 157 days), from each community (bac., bacteria; arc., archaea and fun., fungi) inhabiting each habitat (surface and hyporheic sediment and buried leaves). The shaded area indicate rewetting phase.

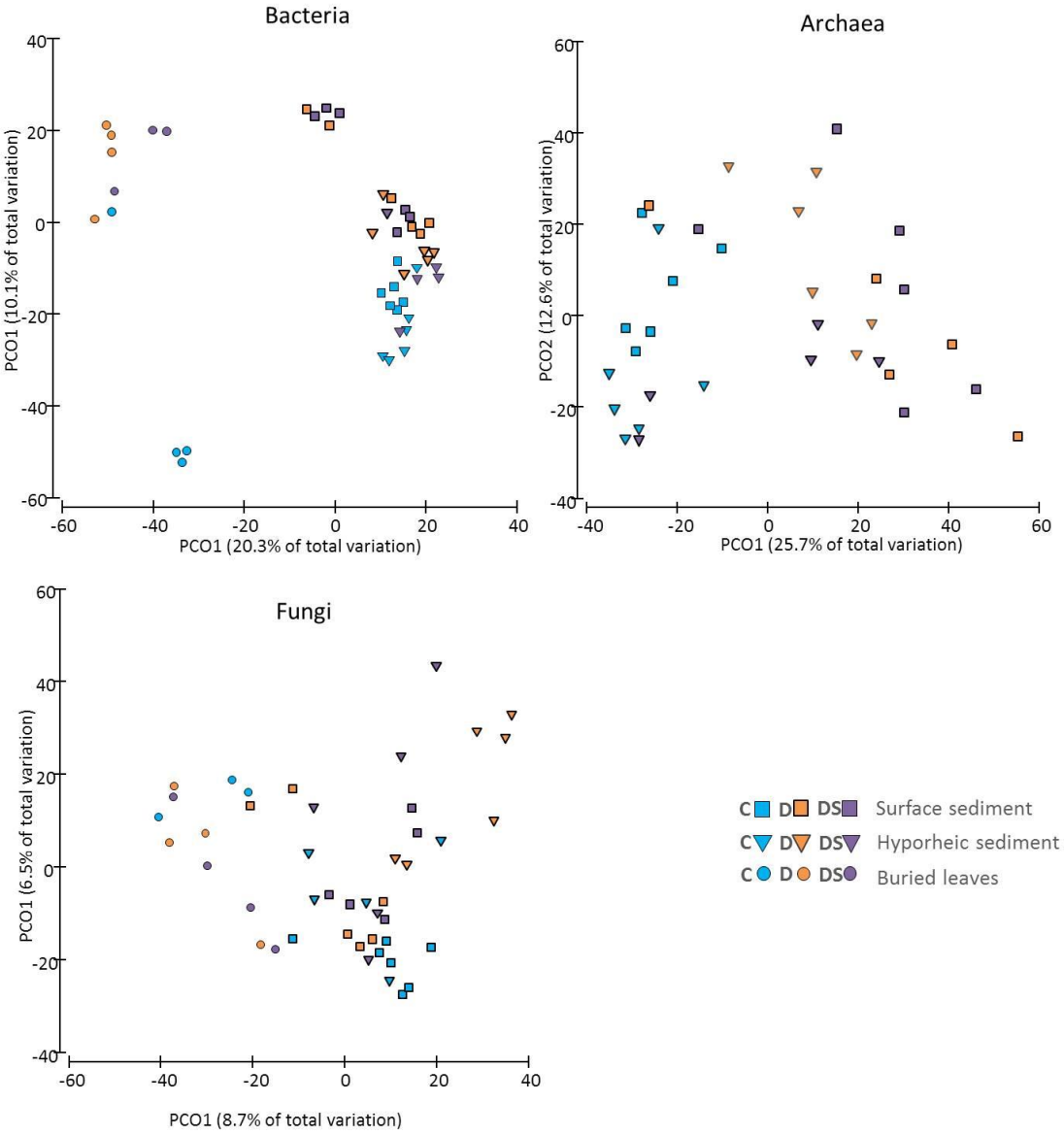


Fig.S3 PCO from bacterial, archaeal and fungal OTUs, inhabiting the three habitats, indicated as: surface sediment; hyporheic sediment; buried leaves. The colour pattern is indicated in the legend for the three treatments: C, Control; D, Dry; DS, Dry-Storm.

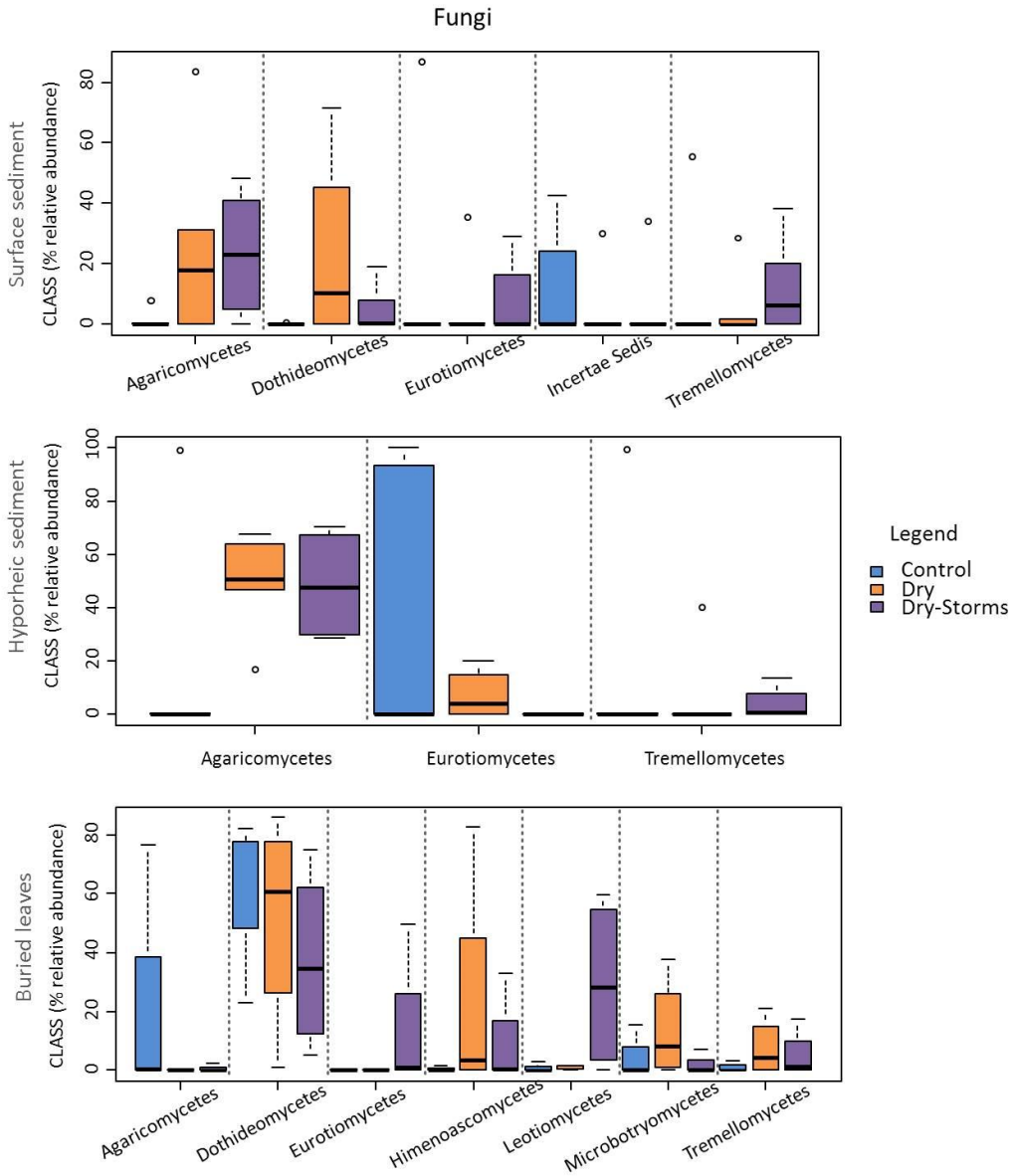


Fig.S4 Boxplots for fungal classes variability (% of relative abundance) considering the whole experimental period. The three habitats are indicated as: surface sediment; hyporheic sediment; buried leaves. The colour pattern is indicated in the legend for the three treatments: C, Control; D, Dry; DS, Dry-Storm. Only the classes presenting relative abundances >5% have been considered.

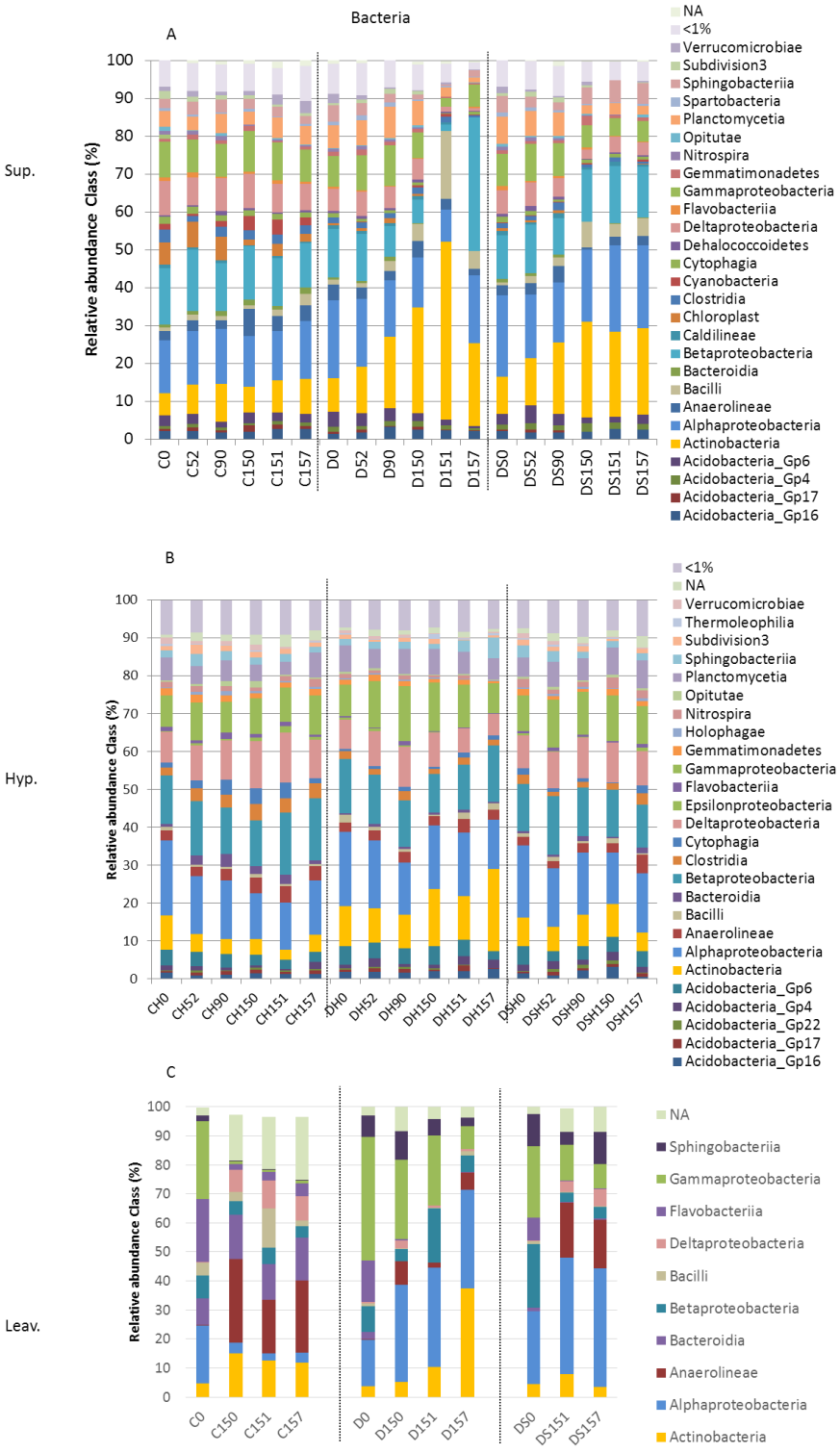


Fig.S5A-B-C Histogram of bacterial class composition (% of relative abundance) inhabiting surface (A) and hyporheic (B) sediment and buried leaves (C). In the *x-axis* the three treatments are indicated (C, D, DS) for each sampling time (day 0, 52, 90, 150, 151, 157).

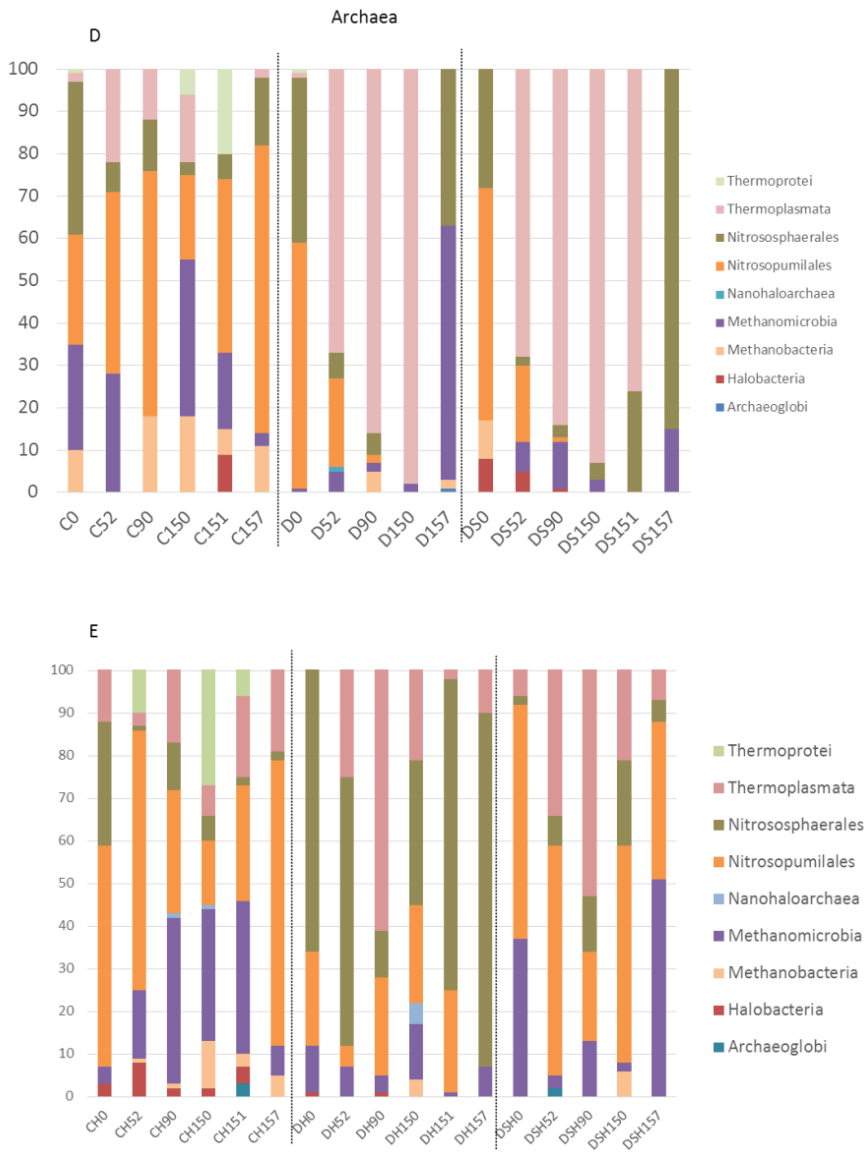


Fig.S5D-E Histogram of archaeal class composition (% of relative abundance) inhabiting surface (D) and hyporheic (E) sediment. In the *x-axis* the three treatments are indicated (C, D, DS) for each sampling time (day 0, 52, 90, 150, 151, 157).

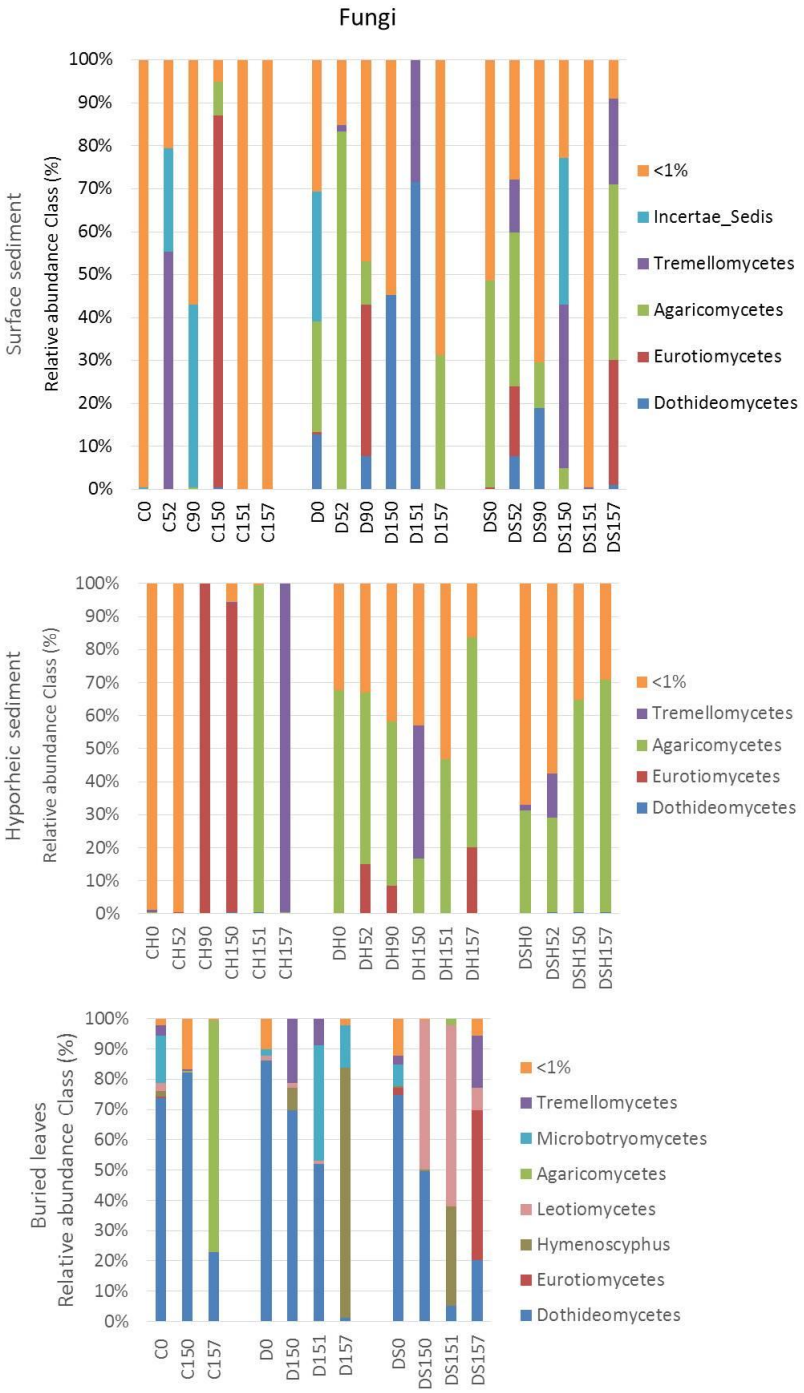


Fig.S6 Histograms of fungal class composition (% of relative abundance) for the three habitats studied, indicated as: surface sediment; hyporheic sediment; buried leaves.In the *x-axis* the three treatments are indicated (C, D, DS) for each sampling time (day 0, 52, 90, 150, 151, 157).

Table S1. A) Dissolved oxygen (DO) in the sediment monitored during the experimental period and the average (±SD) for each treatment and habitat; **B)** Water sediment content (%±SD) assessed at each sampling time. The habitats are indicated with the abbreviations as SUP: surface sediment and HYP: hyporheic sediment whereas the three treatments are stated as C: Control, D: Dry, DS: Dry-Storms.

A

		DO (mg/l)										
Sampling date (day)		*19-feb*	*03-mar*	*17-mar*	*05-apr*	*19-apr*	*04-may*	*24-may*	*25-jun*	*15-jul*	**DO average**	**SD**
	C	3.90	5.75	6.08	5.42	5.51	1.15	1.94	4.03	2.87	4.07	1.68
SUP	D	7.42	8.09	8.87	8.70	8.90	8.74	8.92	8.52	8.68	8.54	0.46
	DS	7.50	8.29	8.81	8.76	8.81	9.67	8.97	8.69	8.86	8.71	0.55
	C	0.08	0.08	0.03	0.06	0.03	0.17	0.04	0.07	0.07	0.07	0.04
HYP	D	7.08	7.78	9.06	8.80	8.86	8.96	8.80	8.48	8.68	8.50	0.61
	DS	4.32	8.32	8.73	8.65	9.02	9.46	9.20	8.24	8.80	8.31	1.46

B

		Water content average (%) ± SD					
Sampling time (day)		*0*	*52*	*90*	*150*	*151*	*157*
	C	17.27±2.19	29.5±0.54	28.48±0.43	29.25±1.29	21.96±1.33	23.86±4.43
SUP	D	15.58±1.55	0.83±0.54	0.34±0.37	0.44±0.91	13.68±1.33	22.71±4.43
	DS	16.57±1.26	1.14±0.54	0.36±0.37	2.73±0.91	12.63±1.33	21.58±4.43
	C	43.29±1.30	27.74±1.51	26.61±0.90	27.45±1.21	20.57±2.11	23.73±2.68
HYP	D	44.71±1.06	4.04±1.06	2.82±0.64	0.65±1.05	18.46±2.11	22.06±2.68
	DS	43.43±1.3	4.55±1.06	2.86±0.64	5.48±1.05	20.01±2.11	20.31±2.68

Table S2. Physicochemical parameters in stream water monitored during the experimental period and further checked with the ACA dataset ("Agència Catalana de l'Aigua" dataset January 2016 - August 2016). During the study period the stream was in base flow conditions and no storms occurred.

	Mean value	**Unit**
N-NO_2	0.05 ± 0.12	mg/L
N-NO_3	0.14 ± 0.12	mg/L
P-PO_4	0.83 ± 0.12	mg/L
DOC	10.43 ± 1.38	mg/L
Water Tº (range)	7 - 15.6	ºC
Dissolved Oxygen	10.18 ± 3.58	mg/L
Conductivity	488 ± 41.39	µS/cm
pH	8.54 ± 0.06	

Suppl. Info. S1. Results from the repeated measures for Shannon-Wiener diversity (H) and Richness (S) indices and for the Unifrac distance (proxy of phylogenetic distance). The acronyms TR, TI and HAB indicate Treatments, Time and Habitats (surface and hyporheic zone), respectively.

BACTERIA - H index			**ARCHAEA - H index**			**FUNGI - H index**		
Origin	F	Sign.	Origin	F	Sign.	Origin	F	Sign.
Intercept	18,598,808	,000	Intercept	1,074,519	,000	Intercept	158,212	,000
TR	3,732	*,062*	TR	5,710	**,029**	TR	4,758	**,044**
TI	9,101	**,002**	TI	1,735	,233	TI	2,949	*,084*
HAB	14,188	**,004**	HAB	8,695	**,018**	HAB	5,242	**,051**
TR * TI	3,105	**,044**	TR * TI	2,117	,150	TR * TI	,623	,763
TR * HAB	1,971	,190	TR * HAB	,250	,785	TR * HAB	3,699	*,073*
TI * HAB	2,507	,101	TI * HAB	3,288	*,066*	TI * HAB	1,633	,256

BACTERIA – S index			**ARCHAEA – S index**			**FUNGI – S index**		
Origin	F	Sign.	Origin	F	Sign.	Origin	F	Sign.
Intercept	7,810,217	,000	Intercept	248,703	,000	Intercept	103,463	,000
TR	1,657	,239	TR	11,250	**,005**	TR	,933	,432
TI	48,502	**,000**	TI	,968	,490	TI	2,400	,130
HAB	19,662	**,001**	DE	5,431	**,048**	HAB	1,796	,217
TR * TI	7,657	**,002**	TR * TI	,754	,669	TR * TI	1,518	,283
TR * HAB	11,459	**,003**	TR * DE	1,892	,212	TR * HAB	2,607	,134
TI * HAB	7,602	**,003**	TI * DE	2,604	,110	TI * HAB	1,548	,277

BACTERIA Unifrac			**ARCHAEA Unifrac**			**FUNGI Unifrac**		
Origin	F	Sign.	Origin	F	Sign.	Origin	F	Sign.
Intercept	2,523,566	,000	Intercept	775,760	,000	Intercept	520,677	,000
TR	14,794	**,001**	TR	,894	,446	TR	1,578	,264
TI	1,553	,259	TI	1,722	,235	TI	,285	,908
HAB	21,557	**,001**	HAB	11,416	**,010**	HAB	1,223	,301
TR * TI	,935	,541	TR * TI	1,127	,441	TR * TI	,600	,779
TR * HAB	14,658	**,001**	TR * HAB	3,875	*,067*	TR * HAB	5,205	**,036**
TI * HAB	,220	,946	TI * HAB	2,000	,183	TI * HAB	,492	,774

BACTERIA - H index	**LEAVES**		**FUNGI - H index**	**LEAVES**
Origin	F	Sign.	Origin	F	Sign.
Intercept	26,234,067	,000	Intercept	80,404	,000
TR	17,734	**,003**	TR	,891	,467
TI	2,529	,154	TI	1,452	,333

BACTERIA – S index	**LEAVES**		**FUNGI – S index**	**LEAVES**
Origin	F	Sign.	Origin	F	Sign.
Intercept	1,675,366	,000	Intercept	33,297	,002
TR	23,995	**,001**	TR	,165	,853
TI	5,374	**,039**	TI	3,766	*,094*

Table S3. Results (p-values) from the PERMANOVA analyses applied over the initial communities (referred to Figure S1). The acronyms SUR and HYP state for Surface and Hyporheic sediment, respectively. The letters C, D and DS state for the three treatments Control, Dry and Dry-Storms respectively.

	BACTERIA			ARCHAEA		FUNGI		
	SUR	HYP	LEAVES	SUR	HYP	SUR	HYP	LEAVES
C, D	0.329	0.662	0.671	0.670	0.332	0.670	0.664	0.663
C, DS	0.335	1.000	0.672	0.669	0.336	0.333	1.000	0.669
D, DS	1.000	0.343	1.000	0.671	0.327	0.666	1.000	0.672

Table S4. Richness (S) and Shannon diversity (H), based on number of OTUs of bacterial, archaeal and fungal assemblages at four dry-phase times (t0, t52, t90, t150) and at two rewetting-phase times (t151, t157), for three different habitats (SUR, surface sediment; HYP, hyporheic sediment; LEAVES, buried leaves) and three treatments (C, Control; D, Dry; DS, Dry-Storms).

			t0		t52		t90		t150		t151		t157	
	Habitat	Treat.	S	H	S	H	S	H	S	H	S	H	S	H
Bacteria	SUR	C	3107.1	6.98	3055.2	7.02	2094.1	6.64	2391.5	6.72	2282.4	6.73	2127.1	6.63
		D	2880.8	7.03	3388.2	7.22	2897.5	7.03	2335.3	6.63	1140.7	4.58	1585.7	5.18
		DS	3317.1	7.15	3886.3	7.35	2931.6	7.00	1807.8	6.08	1962.6	6.18	2348.6	6.46
	HYP	C	3092.7	7.09	3218.8	7.18	3271.0	7.18	2697.5	7.00	2738.1	6.96	2801.2	7.06
		D	2711.5	6.90	3538.4	7.26	3577.8	7.27	3204.7	7.17	1938.1	6.53	2150.8	6.40
		DS	2751.6	6.91	2912.5	7.09	3272.8	7.20	2071.0	6.65	2201.1	6.75	2201.1	6.75
	LEAVES	C	925.1	4.55	na	na	na	na	1041.2	4.50	1125.5	4.80	998.7	4.81
		D	766.0	4.40	na	na	na	na	672.1	4.42	856.0	4.45	489.2	4.35
		DS	964.6	4.67	na	na	na	na	1064.4	4.77	1164.3	4.87	910.4	4.96
Archaea	SUR	C	33.0	2.54	17.0	1.89	11.0	1.92	12.0	2.14	23.5	2.27	21.0	1.40
		D	16.0	1.77	16.5	2.42	19.0	1.99	9.0	1.71	na	na	5.0	0.91
		DS	20.5	1.83	20.0	2.50	16.0	1.84	6.0	0.59	6.0	1.08	12.0	2.01
	HYP	C	18.5	2.10	25.5	2.28	47.5	2.89	29.5	2.94	33.0	2.72	39.0	2.35
		D	14.7	2.09	20.5	1.55	22.0	2.39	16.5	2.55	12.0	1.90	10.0	1.82
		DS	11.0	1.49	17.5	2.31	22.0	2.68	22.2	2.10	na	na	17.0	2.16
Fungi	SUR	C	4.0	0.76	6.0	1.55	8.5	1.55	8.5	0.60	6.0	1.22	3.0	0.58
		D	7.0	1.57	5.0	0.96	14.0	2.30	6.0	1.12	3.0	0.63	3.0	0.74
		DS	7.0	1.39	16.2	2.45	9.0	1.63	5.0	1.30	5.0	1.35	11.0	1.76
	HYP	C	8.0	0.76	4.0	1.55	1.0	1.55	9.0	0.60	4.5	1.22	2.0	0.58
		D	5.0	1.57	18.5	0.96	7.0	2.30	5.0	1.12	6.0	0.63	7.0	0.74
		DS	6.0	1.39	12.5	2.45	na	1.63	3.0	1.30	na	1.35	3.0	1.76
	LEAVES	C	31.5	1.76	na	na	na	na	25.0	1.02	na	na	6.0	0.68
		D	21.7	1.53	na	na	na	na	46.0	2.14	7.0	1.45	5.0	0.58
		DS	19.3	1.68	na	na	na	na	22.0	1.84	6.0	1.06	17.0	1.75

Table S5. *p*-values from PERMANOVA analyses from complete PCO (Fig. S3). Differences between *Treatments* (C, D, and DS), type of *Habitats* (surface and hyporheic sediment, buried leaves) and the interaction *Treatment* x *Habitats* were assessed for each microbial community (bacteria, archaea, fungi). p-values indicating significant difference ($p<0.05$) are indicated in boldface while p-values at the limit of significance ($p<0.1$) are indicated in italic.

	Bacteria	**Archaea**	**Fungi**
Treatment	**<0.001**	**<0.001**	0.516
Habitats	**<0.001**	**0.025**	**<0.001**
Treatment x Habitats	**0.003**	*0.080*	**0.047**

CHAPTER 3

Fig. S1 Results of principal coordinated analyses of distribution for A) DNA prokaryotes B) RNA prokaryotes C) DNA eukaryotes D) RNA eukaryotes E) CLPPs

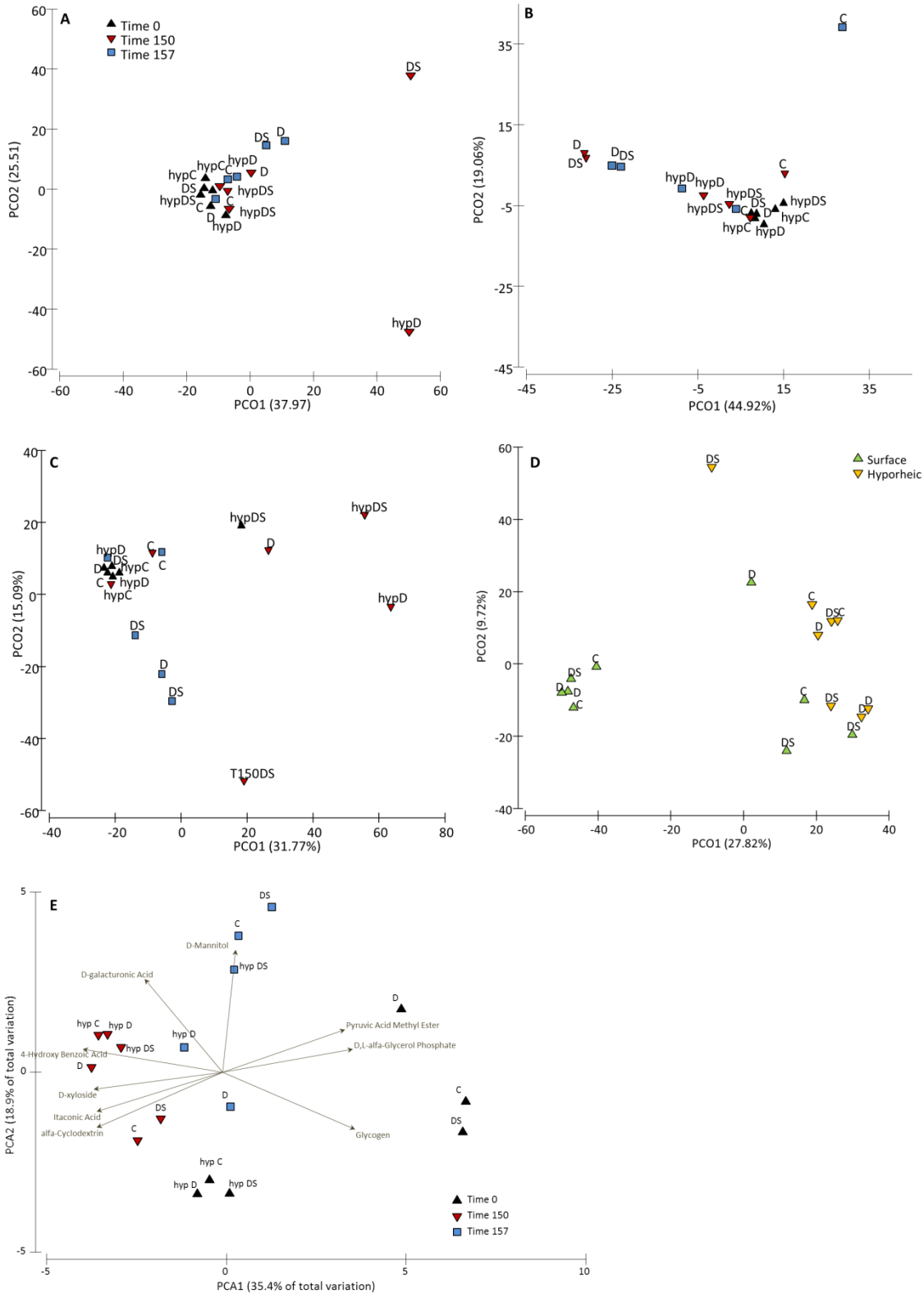


Table S1 Relative abundance of A) Prokaryotes phyla B) Eukaryotes groups

A) Phyla
Prokaryotes

SURFACE	T0 C	T150 C	T157 C	T0 DS	T150 DS	T157 DS	T0 D	T150 D	T157 D
Euryarchaeota	0.00	0.00	0.00	0.00	0.96	0.28	0.00	1.34	0.54
Thaumarchaeota	0.22	0.13	0.00	0.34	0.49	0.26	0.00	0.33	0.34
Acetothermia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.09	0.00
Acidobacteria	4.73	2.85	3.71	5.59	1.51	3.83	5.90	0.31	0.87
Actinobacteria	3.00	2.03	3.27	2.86	20.28	8.09	2.93	43.14	7.32
Bacteroidetes	6.11	3.65	4.66	6.57	6.67	4.65	8.89	3.48	12.33
Chloroflexi	1.71	1.05	3.51	1.45	0.66	1.12	1.38	1.13	0.66
Cyanobacteria	0.70	17.89	10.45	0.30	1.62	3.36	1.20	1.06	2.02
Firmicutes	0.26	0.35	0.68	0.08	16.18	9.32	0.70	3.48	13.82
Gemmatimonadetes	0.69	0.25	0.07	0.77	0.01	0.01	0.90	0.01	0.00
Nitrospinae	0.12	0.63	0.87	0.08	0.00	0.00	0.12	0.00	0.00
Nitrospirae	2.87	2.17	1.63	4.36	0.27	1.12	3.49	0.10	0.17
Planctomycetes	0.97	0.79	2.84	1.04	0.01	0.43	1.73	1.03	0.01
Proteobacteria	72.98	63.71	61.64	70.77	50.50	64.02	67.23	39.36	60.76
Tectomicrobia	3.79	2.72	1.59	4.33	0.44	2.38	4.02	1.97	0.63
Verrucomicrobia	1.72	1.30	2.99	1.39	0.41	0.65	1.53	2.16	0.50
HYPORHEIC	T0 C	T150 C	T157 C	T0 DS	T150 DS	T157 DS	T0 D	T150 D	T157 D
Euryarchaeota	0.00	0.00	n.a.	0.35	0.96	0.94	0.00	5.34	0.90
Thaumarchaeota	0.49	0.30	n.a.	0.51	0.97	0.42	0.78	1.27	0.40
Acetothermia	0.00	0.00	n.a.	0.00	0.00	0.00	0.00	0.00	0.00
Acidobacteria	4.58	5.11	n.a.	3.61	4.31	4.27	3.47	2.54	2.99
Actinobacteria	3.84	3.84	n.a.	2.41	4.34	3.40	3.29	17.31	7.61
Bacteroidetes	5.03	4.77	n.a.	4.17	2.30	1.35	4.69	0.65	5.13
Chloroflexi	2.13	2.86	n.a.	1.10	1.64	1.31	1.42	4.01	1.69
Cyanobacteria	1.21	0.83	n.a.	0.34	0.09	0.95	5.24	0.15	0.90
Firmicutes	1.04	0.85	n.a.	0.57	0.65	0.35	1.37	0.59	3.20
Gemmatimonadetes	0.59	0.55	n.a.	0.59	0.64	0.38	0.37	0.26	0.10
Nitrospinae	0.06	0.14	n.a.	0.93	0.16	0.21	0.08	0.15	0.19
Nitrospirae	3.32	4.02	n.a.	8.70	3.91	4.47	3.09	1.59	0.80
Planctomycetes	0.99	0.80	n.a.	0.89	1.49	1.51	0.71	0.64	0.35
Proteobacteria	71.73	69.78	n.a.	68.96	70.31	73.38	70.50	58.39	72.32
Tectomicrobia	3.74	4.81	n.a.	3.49	5.64	5.48	3.59	4.66	1.86
Verrucomicrobia	1.12	1.12	n.a.	1.31	2.43	1.47	1.30	2.43	1.45

B) Eukaryotes

SURFACE	T0 C	T150 C	T157 C	T0 DS	T150 DS	T157 DS	T0 D	T150 D	T157 D
Ambiguous_taxa	0.22	0.36	0.00	0.00	0.00	0.00	0.08	0.12	0.00
Apicomplexa	0.16	1.41	0.00	0.00	0.38	0.00	0.15	0.42	0.00
Bicosoecida	0.00	1.21	0.00	0.00	1.24	0.00	0.00	0.00	0.00
Cercozoa	8.75	8.39	0.00	0.05	4.52	0.00	13.68	8.83	7.02
Chlorophyta	1.16	0.40	0.00	0.00	0.04	0.00	2.50	0.53	0.00
Choanoflagellida	0.87	1.57	0.00	0.00	5.45	0.00	0.04	0.02	0.00
Ciliophora	9.87	48.73	0.00	0.00	72.56	0.00	18.74	10.66	43.08
Dictyamoeba	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Dinoflagellata	0.61	2.54	0.00	0.00	0.00	0.00	0.12	0.16	0.00
Discicristata	0.60	1.04	0.00	0.00	0.45	0.00	0.71	1.01	0.00
Discicristoidea	0.00	0.08	0.00	0.00	0.01	0.00	0.87	0.37	0.00
Euamoebida	0.01	1.06	0.00	0.00	0.06	0.00	0.01	0.00	0.00
Flabellinia	0.29	3.31	0.00	18.37	1.11	0.00	0.04	0.02	0.00
Flamella	0.00	0.82	0.00	0.00	0.43	0.00	0.01	0.02	0.00
Florideophycidae	0.65	0.00	0.00	0.00	0.01	0.00	0.07	0.05	0.00
Fungi	3.45	6.24	99.93	3.22	6.63	99.92	7.79	5.24	44.41
Hyphochytriomycetes	0.00	0.69	0.00	0.00	0.29	0.00	0.01	0.05	0.00
Ichthyosporea	0.00	0.00	0.00	0.00	0.01	0.00	0.22	1.09	0.00
Ischnamoeba	0.24	1.12	0.00	0.00	0.56	0.00	0.17	0.15	0.00
Jakobida	0.00	1.34	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Leptomyxida	0.06	1.43	0.00	0.00	0.03	0.00	0.00	0.02	0.00
Ochrophyta	71.71	17.15	0.07	78.35	3.99	0.08	54.64	71.08	5.47
Peronosporomycetes	1.33	1.07	0.00	0.00	2.21	0.01	0.13	0.14	0.00
HYPORHEIC	T0 C	T150 C	T157 C	T0 DS	T150 DS	T157 DS	T0 D	T150 D	T157 D
Ambiguous_taxa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Apicomplexa	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00
Bicosoecida	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00
Cercozoa	31.25	38.44	0.02	1.76	0.06	1.36	0.00	0.00	5.00
Chlorophyta	0.00	0.00	0.00	0.59	0.00	13.38	0.00	0.00	5.00
Choanoflagellida	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00
Ciliophora	18.75	0.02	0.08	2.94	0.02	21.33	0.00	0.09	5.00
Dictyamoeba	0.00	0.00	0.00	0.00	0.00	6.11	0.00	0.00	0.00
Dinoflagellata	0.00	0.00	0.00	0.59	0.02	2.72	0.00	0.00	0.00
Discicristata	0.00	0.00	0.00	0.59	0.00	2.79	0.00	0.00	0.00
Discicristoidea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Euamoebida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Flabellinia	0.00	0.00	0.00	0.00	0.02	12.64	1.08	0.00	0.00
Flamella	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Florideophycidae	0.00	0.00	0.04	0.00	0.00	10.73	0.00	0.00	0.00
Fungi	6.25	61.49	99.78	87.65	99.71	13.25	96.77	32.70	40.00
Hyphochytriomycetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ichthyosporea	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00

Ischnamoeba	0.00	0.00	0.02	0.59	0.02	5.57	0.00	67.19	0.00
Jakobida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
Leptomyxida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ochrophyta	43.75	0.05	0.04	2.35	0.13	10.12	2.15	0.01	45.00
Peronosporomycetes	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00

CHAPTER 4

Supplementary information S1: Detailed hydrological measurements

Specifically, we used the daily variation in streambed and air temperatures to identify, for each sampling site the water presence in the streambed, which is strongly related to its temperature (Constantz et al., 2001; Stromberg et al., 2005). The temperature transducers (Solinst Levelogger 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.

References

Arias-Real R, Menéndez M, Abril M, Oliva F, Muñoz I (2018) Quality and quantity of leaf litter: Both are important for feeding preferences and growth of an aquatic shredder. *Plos One*, 13, e0208272.

Constantz J, Stonestrom D, Stewart AE, Niswonger R, Smith TR. (2001). Analysis of streambed temperatures in ephemeral channels to determine streamflow frequency and duration. *Water Resour Res* 37:317–28.

Colls, M., X. Timoner, C. Font, S. Sabater, and V. Acuña. (2019). Effects of Duration, Frequency, and Severity of the Non-flow Period on Stream Biofilm Metabolism. *Ecosystems*

Stromberg JC, Bagstad KJ, Leenhouts JM, Lite SJ, Makings E. (2005). Effects of stream flow intermittency on riparian vege- tation of a semiarid region river (San Pedro River, Arizona). *River Res Appl* 21:925–38.

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															
Besos	BE6	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
		2°16'11.98"E															
	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															
Foix	FO8	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
		1°30'26.44"E															
	FO11	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															
Tordera	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															
	TO14	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
		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
		2°35'17.97"E															
	TE18	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
		2°13'11.86"E															
	TE19	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
		2°24'38.68"E															
	TE20	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
		2°32'28.68"E															
	TE21	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
		2°20'19.50"E															
	TE22	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
		2°10'21.70"E															
	TE24	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															
	TE25	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
		2°29'20.27"E															
	TE26	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
		2°42'41.04"E															
Francoli	FR27	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															
Fluvia	FL32	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
		2°38'26.65"E															
	FL33	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
		2°26'29.72"E															
	FL34	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
		2°26'53.48"E															
Muga	MU36	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
		2°42'13.25"E															
	MU37	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
		3° 3'6.24"E															
	MU38	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
		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															

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

Supplementary Information S3: 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

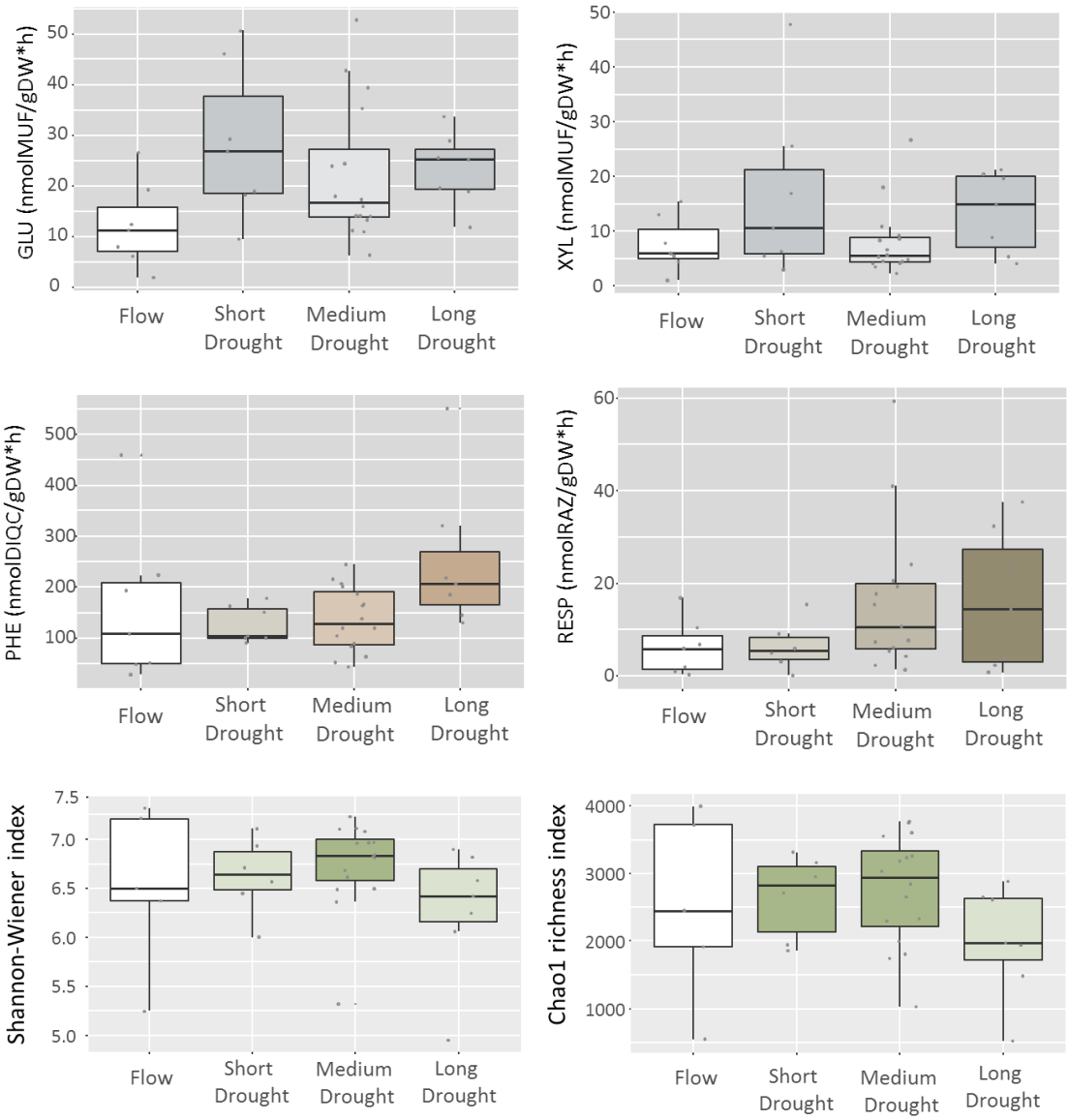


Fig.S1 Boxplots describing values of extracellular enzyme activities (GLU, XYL, PHE), community respiration (RESP) and diversity indices (Shannon and Richness index) in the four hydrological groups considered in the study (FL, SD, MD and LD sites).

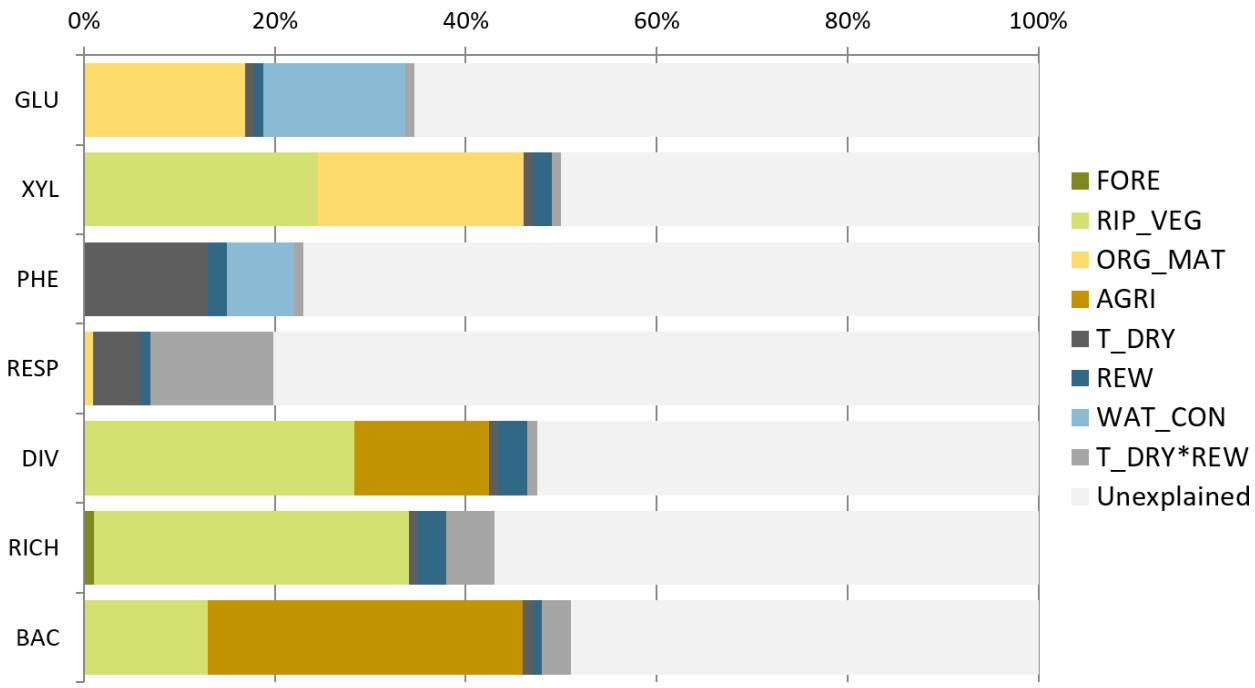


Fig.S2 Variance partitioning results obtained for the averaged models created for each functional (GLU, β-glucosidase; XIL, β-xilosidase; PHE, phenol-oxidase; RESP, community respiration) and structural (DIV, diversity index; RICH, richness index; BAC, bacterial density) microbial community descriptor. The hydrological and environmental factors considered in the models are listed in the legend: forested land-use (FORE); riparian vegetation cover (RIP_VEG); organic matter content (ORG_MAT); agricultural land-use (AGRI); total dry phase duration over the 8-months (T_DRY); duration of the last rewetting phase (REW); sediment water content (WAT_CON); total dry-phase and rewetting interaction (T_DRY*REW).

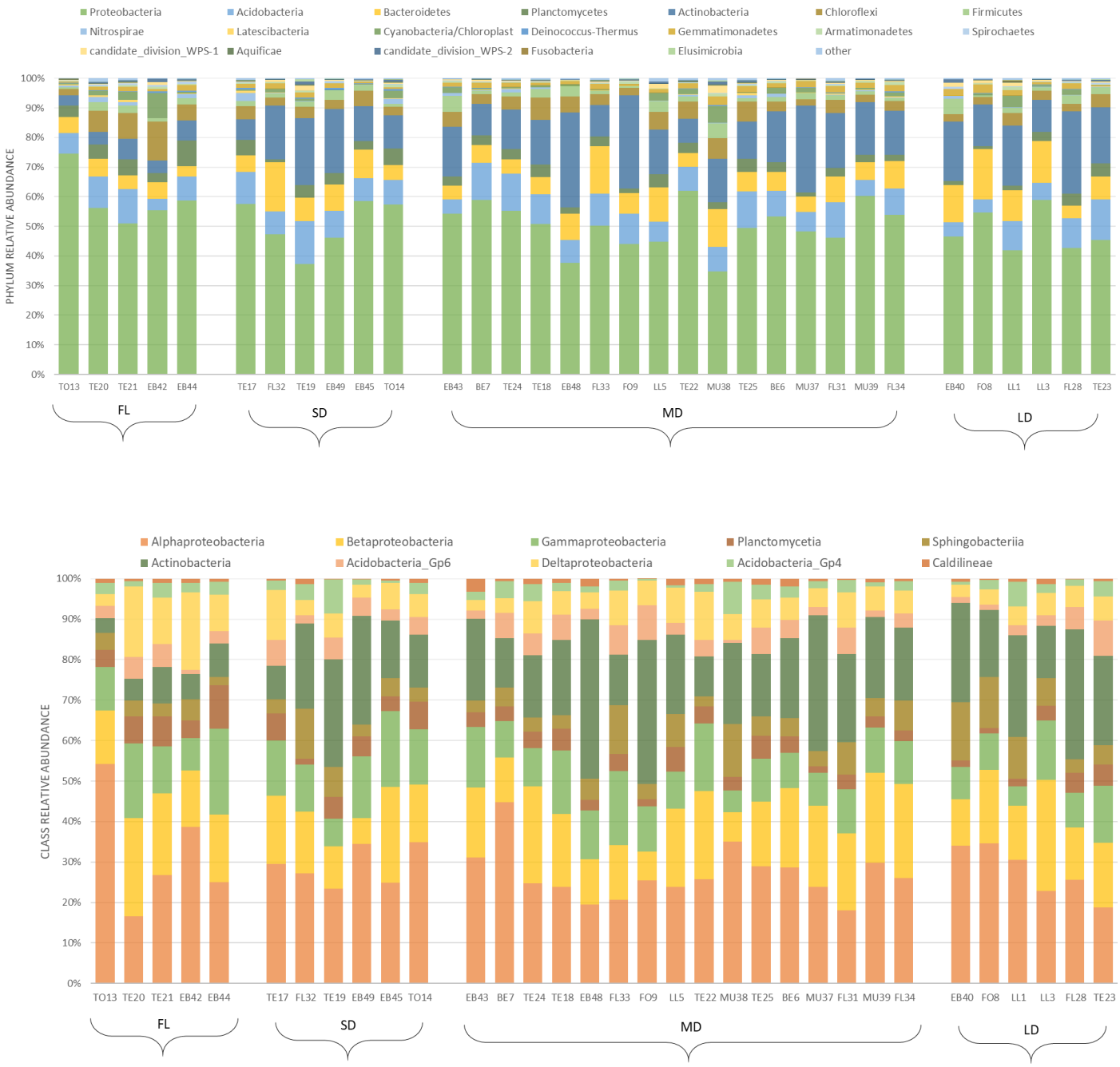


Fig.S3 Bacteria phyla and classes composition. Values are expressed as percentage of relative abundance (%) and separated in the hydrology groups: FL, flow sites; SD, short dry-phase sites; MD, medium dry-phase sites; LD, long dry-phase sites.

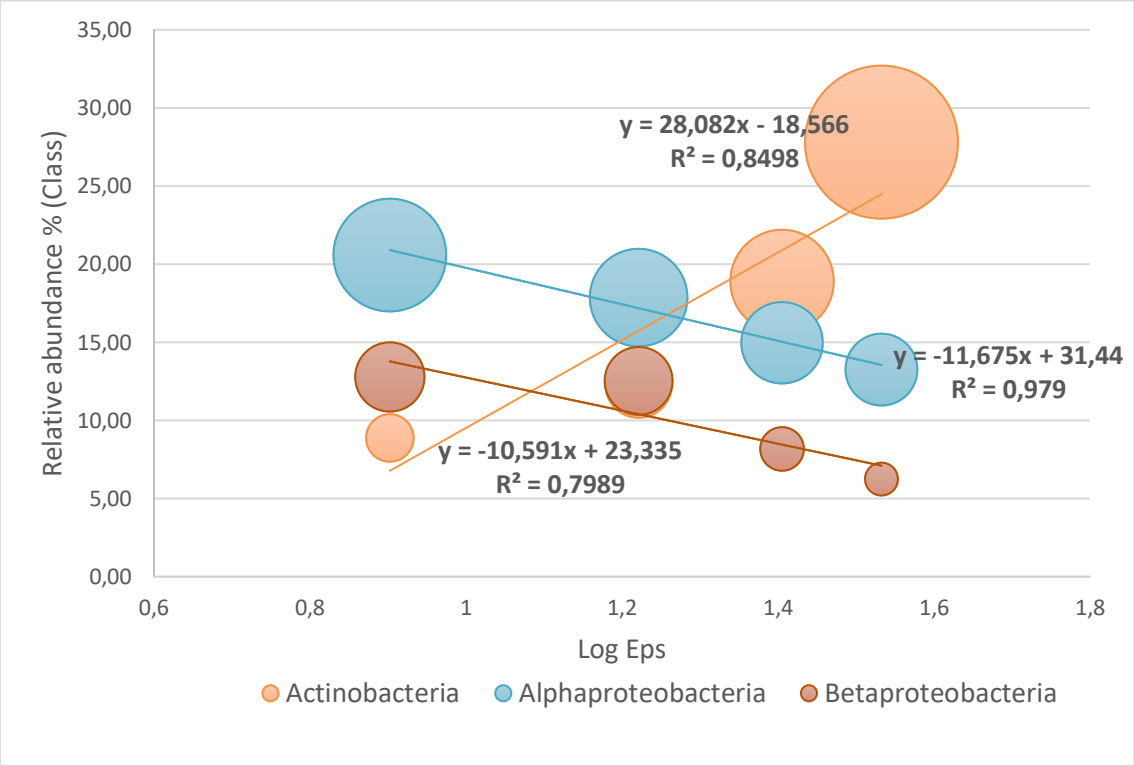


Fig. S1 Relationship between the relative abundance (%) of the Actinobacteria, Alfa- and Beta-proteobacteria taxonomic classes with the extracellular polymeric substances production (EPS), log transformed. The relative abundances resulted from the bacterial community inhabiting the surface sediment during the dry phase.