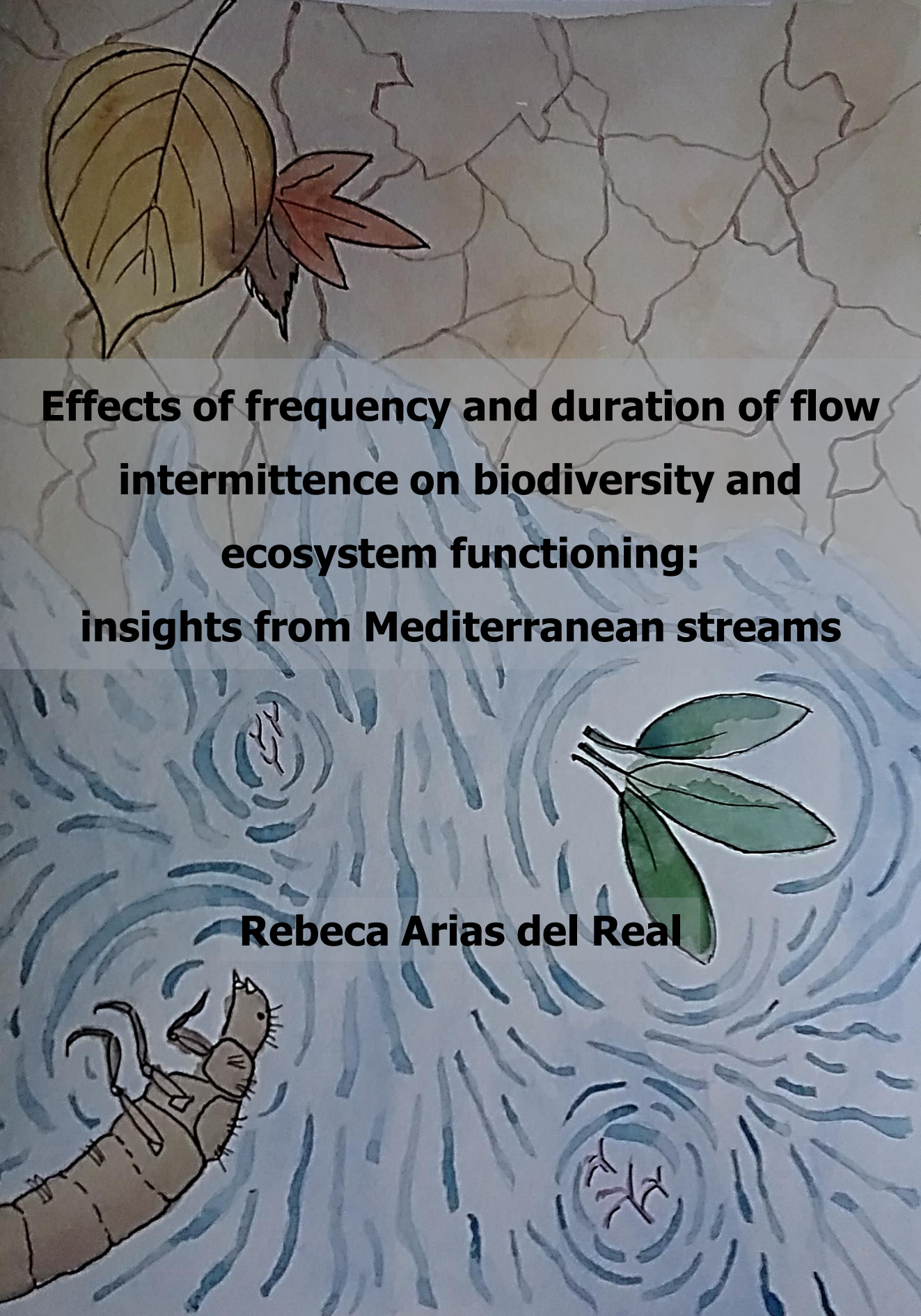
UNIVERSITAT DE BARCELONA

Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights form Mediterranean streams

Rebeca Arias del Real



Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights from Mediterranean streams

Rebeca Arias del Real

UNIVERSITAT DE BARCELONA

Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights from Mediterranean streams

Rebeca Arias del Real

Arias-Real, R. Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights from Mediterranean streams. Doctoral thesis. Universitat de Barcelona, Barcelona, Spain.

Thesis cover by Pilar Hurtado

Covers of chapters 1, 2, 3 and 4 by Pilar Hurtado

All diagrams and schemes by Rebeca Arias del Real

All photographs taken by Rebeca Arias del Real

Invertebrate drawings: Pau Fortuño Estrada. Not to scale

Doctoral Thesis

Department of Evolutionary Biology, Ecology and Environmental Sciences

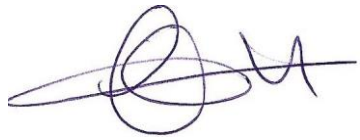
Doctoral program in Ecology, Environmental Sciences and Plant Physiology

Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights from Mediterranean streams

Dissertation presented by Rebeca Arias del Real to apply for the doctoral degree by the University of Barcelona

Rebeca Arias del Real

Barcelona, 2020



Approval of the directors of the doctoral thesis:

Dr. Isabel Muñoz Gracia

Dr. Margarita Menéndez López

A mi familia,

y a ti.

AGRADECIMIENTOS

Rebeca Arias del Real held a predoctoral grant (BES-2015-073961, FPI) from the Ministry of Economy, Industry and Competitiveness of Spain. Futhermore, this study was supported by the project FUNSTREAM (CGL2014-58760-C3-1-R) and SPACESTREAM (CGL2017-88640-C2-2-R) of the Spanish Ministry of Economy, Industry and Competitiveness.

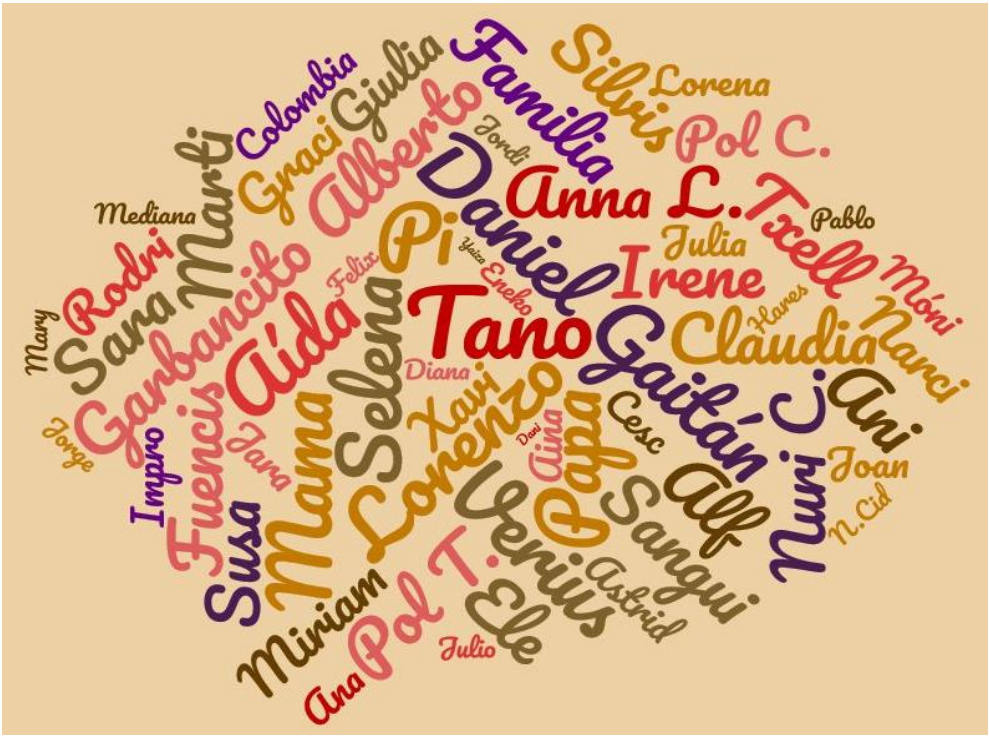
.. ..

El final ha llegado. Ha sido un periodo muy intenso en mi vida, con subidas y bajadas; son muchas historias y situaciones inolvidables, ahora llegó el momento de la despedida o mejor, un hasta pronto….

Mi agradecimiento a las culpables de esta aventura, Isabel y Margarita. Muchas gracias por confiar en mí y darme la oportunidad de realizar un sueño, sobre todo por vuestra paciencia y apoyo infinito. Gracias a vosotras he podido realizar este episodio de mi vida. ¡¡Sin duda no podía haber tenido más suerte!!! No sólo sois unas grandes profesionales, sino que sois excelentes personas.

No puedo despedirme sin agradecer a todas las personas que han pasado por mi vida durante estos años, y a las que siempre han estado a mi lado y que me han apoyado y aguantado todos los vaivenes emocionales que conlleva esta hermosa etapa ….

En ella aprendes conocimientos que te apasionan y también a conocerte a ti mismo y llegar a límites insospechados. Todo ello no habría sido posible sin vosotros, mil gracias por todo, os quiero mucho.



Barcelona, 2020

ADVISORS`REPORT

Dr. Isabel Muñoz and Dr. Margarita Menéndez, professors in the Department of Evolutionary Biology, Ecology and Environmental Sciences (Ecology section), at the University of Barcelona, as supervisors of the Doctoral Thesis presented by Rebeca Arias del Real entitled: ***Effects of frequency and duration of flow intermittence on biodiversity and ecosystem functioning: insights from Mediterranean streams,***

INFORM,

That the research studies developed by Rebeca Arias del Real for her Doctoral Thesis have been organized in four chapters, which corresponds to four scientific papers, two already published, other under review and the last in preparation and will be sent in the next months.

The list of the published chapters, indicating the Journal Impact Factor according to SCI of ISI Web of Science:

Arias-Real, R., Muñoz, I., Gutiérrez-Cánovas, C., Granados, V., Lopez-Laseras, P. and Menéndez, M. 2020. Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period. *Science of the Total Environment*, 703, 135485.

DOI: 10.1016/j.scitotenv.2019.135485.

The impact factor of Science of the Total Environment in 2018 was 5.589. This journal is included in the category of Environmental Sciences and is reported in the First Quartile being in the 27th position of the 250 journals included.

Arias-Real, R., Menéndez, M., Abril, M., Oliva, F. and Muñoz, I. 2018. Quality and quantity of leaf litter: Both are important for feeding preferences and growth of an aquatic shredder. *PLoS ONE*, 13(12): e0, 1-17.

DOI: 10.1371/journal.pone.0208272.

The impact factor of PLoS ONE in 2018 was 2.776. This journal is included in the category of Agricultural and Biological Sciences and is reported in the First Quartile being in the 34th position of the 272 journals included.

The manuscripts under review is:

Arias-Real, R., Gutiérrez-Cánovas, C., Menéndez, M., Granados, V. and Muñoz, I. 2020. Annual and recent flow-intermittence disturbances explain the high variability of stream invertebrate diversity. *Journal of Applied Ecology*.

The impact factor of Journal of Applied Ecology in 2018 was 5.78. This journal is included in the category of Ecology and is reported in the First Quartile being in the 14th position of the 164 journals included.

The manuscripts in preparation is:

Arias-Real, R., Muñoz, I., Pascoal, C., Gutiérrez-Cánovas, C. and Menéndez, M. Flow intermittence drives aquatic hypomycete turnover and richness reduction which affects organic matter decomposition.

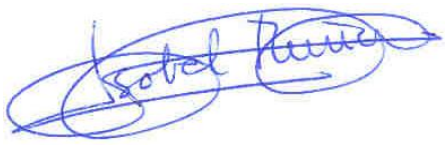
And CERTIFY,

That Rebeca Arias del Real has participated actively on the development of the research and the elaboration associated to each of the papers listed. In particular, her contribution included the following tasks:

- Participation in setting objectives and in the experimental design of each one of the chapters developed
- Sampling design and field work
- Sample processing and analyses in laboratory
- Statistical data analyses and interpretation of results
- Writing, reviewing and editing processes of the manuscripts

Finally, we certify that the co-authors of the papers that conforms this Doctoral Thesis, will not use any of the manuscripts in other Doctoral Thesis.

Barcelona, 5th May 2020



Isabel Muñoz Gracia

Margarita Menéndez López

ABSTRACT

Intermittent rivers and ephemeral streams (IRES) are watercourses that naturally and periodically cease to flow. They represent more than half of the global river network and are expanding due to global change. In this thesis, I investigate the mechanisms linking flow intermittence with biodiversity and ecosystem functioning, which sustain biogeochemical cycles and energy transfer in the system. Chapter 1 analyzes the effects of hydrology, micro-habitat (surface and subsurface zones) and biotic features on organic matter decomposition and fungal biomass, in 20 streams. In Chapter 2, I assess the effect of different flow intermittence metrics (i.e., annual intermittence regime and recent aquatic status) on aquatic biodiversity, including both taxonomic and functional-trait-based metrics, in 33 streams. Chapter 3 analyzes how aquatic hyphomycete richness and composition (beta diversity and its turnover and nestedness components) are affected by a flow intermittence gradient and how these community changes affect organic matter decomposition, in 15 streams and in a microcosm approach. Finally, in Chapter 4, I explore how changes in both leaf litter quality and quantity determine the feeding preferences and growth of an invertebrate shredder.

The results of Chapter 1 show that the subsurface zone contributes to maintaining microbial decomposition during non-flow periods in IRES, mainly because of the levels of fungal biomass present in the subsurface zone. In Chapter 2, I conclude that a combination of flow intermittence metrics are needed to explain the high dynamism of the invertebrate community in IRES and potentially ecosystem functioning. Moreover, this chapter shows that hydrological variables outweigh non-hydrological factors in explaining invertebrate community variation, thereby supporting the use of the former

in IRES classification and bio-monitoring routines. Chapter 3 reveals that the reduction of aquatic hyphomycete richness and species turnover as a result of flow intermittence, could have negative effects on organic matter decomposition. Finally, in Chapter 4, I provide evidence on how flow intermittence reduces the quality of leaf litter, in terms of fungal richness and composition, fungal biomass and lipid content. These changes in food quality influence the consumption rates and growth of shredders, which are able to feed selectively on higher quality leaves, even though its availability is lower. Taken together, these results will help to improve the biomonitoring and management of IRES and to a better prediction of ecosystem trajectories in response to global change.

CONTENTS

LIST OF FIGURES

General Introduction

Research Objectives

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Annex (Supplementary material)

Chapter 1

Chapter 2

LIST OF TABLES

General Introduction

Chapter 1

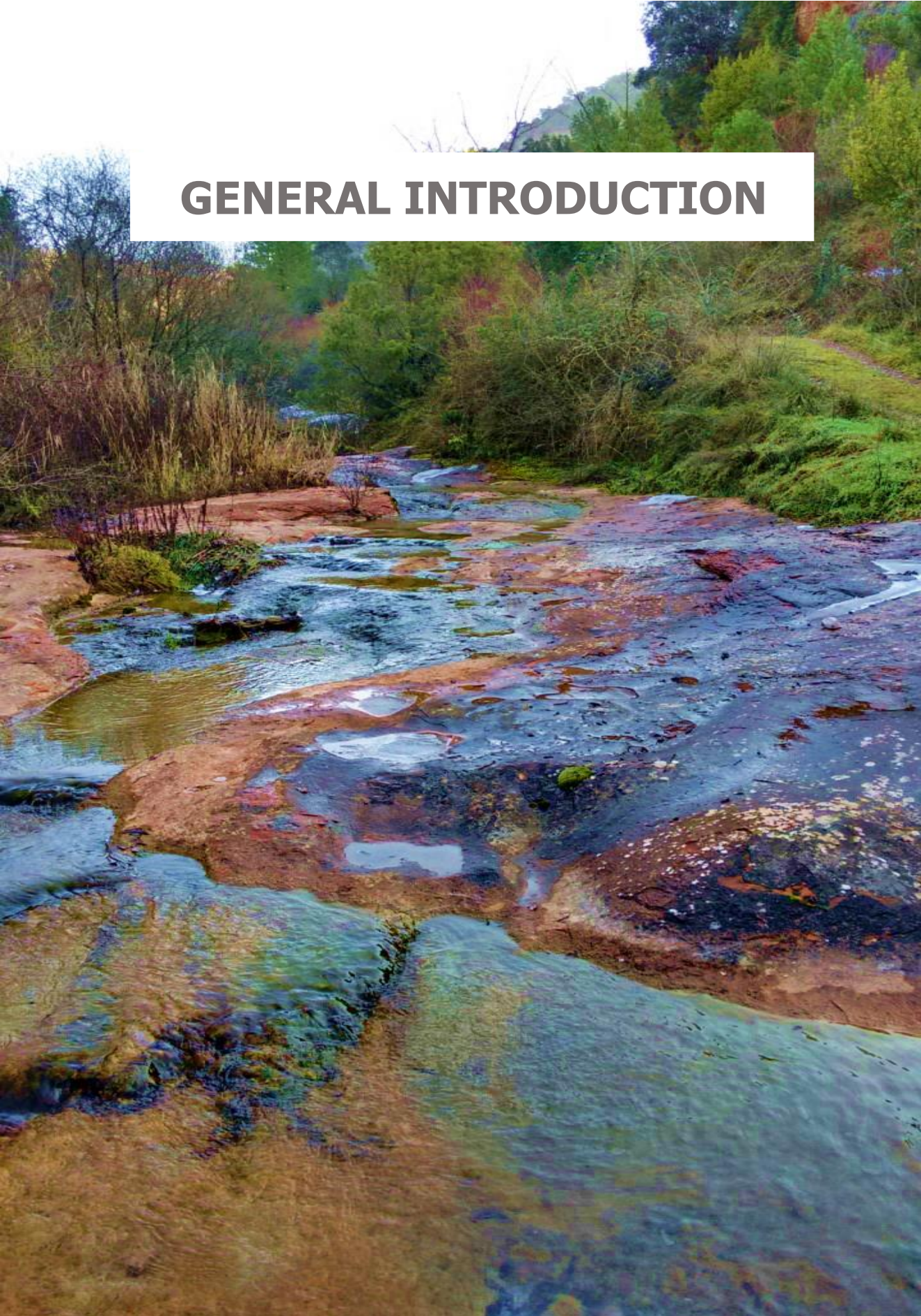
Chapter 2

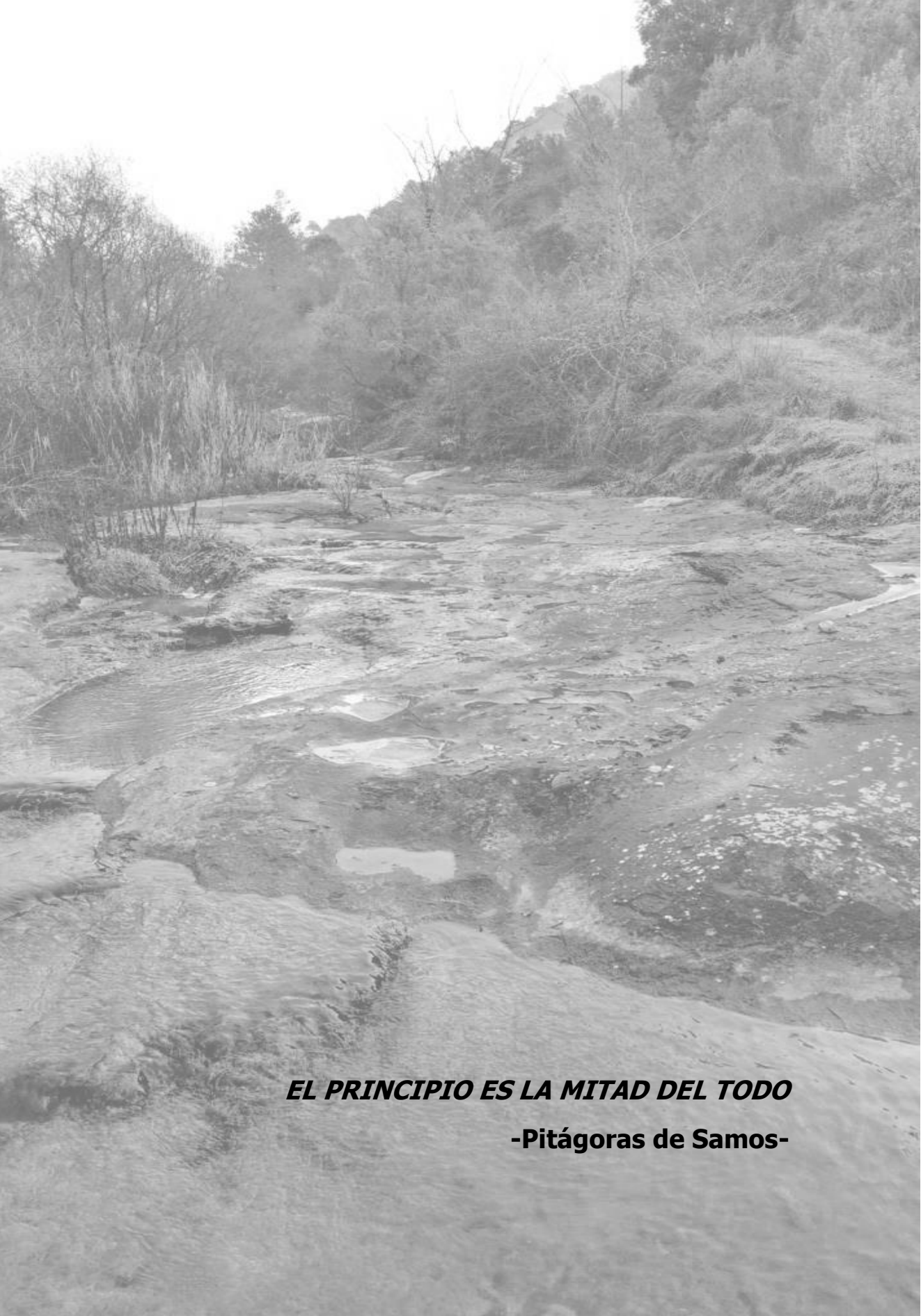
Chapter 2

Chapter 3

Chapter 4

GENERAL INTRODUCTION





EL PRINCIPIO ES LA MITAD DEL TODO

-Pitágoras de Samos-

GENERAL INTRODUCTION

The presence of an active flow plays a central role in shaping biophysical dynamics, structure and functioning of fluvial ecosystems, controlling crucial aspects such as geomorphology and the quality of both the water and the overall habitat. Moreover, flow activity establishes hydrological connectivity along the watercourse as defined by Pringle (Pringle, 2003): "*water-mediated transfer of matter, energy, or organisms within and/or between elements of the hydrologic cycle*". Hydrological connectivity is characterized by three spatial dimensions longitudinal, lateral and vertical that interact over a fourth dimension –time- (Ward, 1989). The spatial dimensions are not restricted to water, since nutrients, dissolved and particulate organic matter, sediments, and aquatic organisms also move through them in watercourse (Rolls *et al.*, 2018). Longitudinally, water, sediments, organic matter and biota are transported downstream, contributing to the physicochemical characterization of the water and the availability of inorganic and organic matter for organisms (Freeman *et al.*, 2007). Rivers are connected laterally with the riparian zone that contributes to the allochthonous inputs that fuels riverine communities (Sanpera-Calbet *et al.*, 2016). Meanwhile, vertically, the river is connected with the hyporheic zone and groundwater which is biochemically highly dynamic and can act as a refuge for organic matter, microbes and invertebrates (Boulton *et al.*, 1998).

Rivers are ecosystems that fluctuate considerably, due to seasonal variations in flow, light and temperature, which depend on catchment morphology, lithology and climate. In the most extreme cases, some rivers recurrently

cease to flow in time and/or space, and may eventually dry up. This begs us to enquire: What happens when flow ceases or rivers dry up? What happens to the hydrological connectivity? What happens to aquatic organisms and ecological functions they support? **(Figure 1)**.

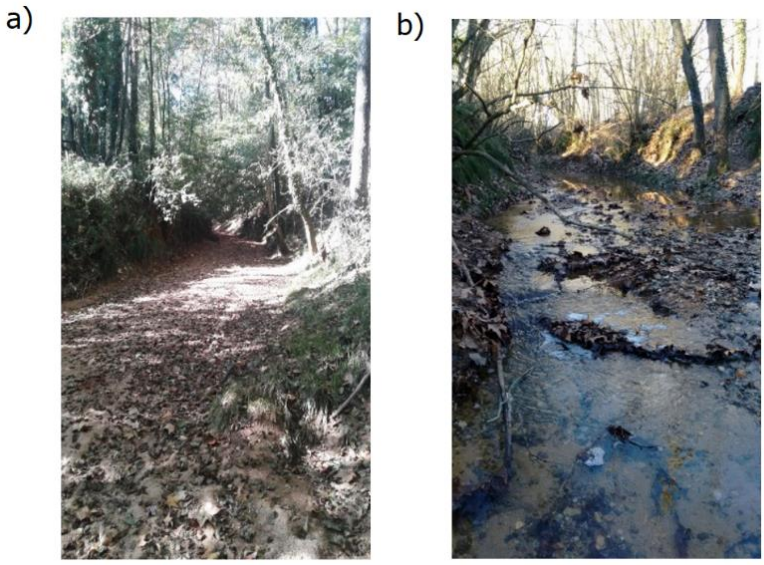


Figure 1. Onyar stream (NE Spain) in: October 2016 (a) and February 2017 (b).

Intermittent rivers and ephemeral streams

Intermittent rives and ephemeral streams (hereinafter: IRES) are those that naturally experience occasional periods of complete flow disruption, in time or space (Datry *et al.*, 2017b). Currently, this type of watercourse represents roughly half of the global drainage network; yet this

estimation is rather conservative, as some low-order stream are difficult to detect and map, so they could make up more than 70% of the drainage network (Snelder *et al.*, 2013).

IRES are widely spread across regions that experience a hydric deficit during the dry season (e.g., hyper-arid, arid, semi-arid and dry sub-humid climates) or because water is in its solid state due to low temperatures (alpine, Arctic and Antarctic climates) (Skoulikidis *et al.*, 2017). Even in wet temperate and tropical areas, headwaters can consist of IRES that are tightly linked to rainfall events (Caruso & Haynes, 2011). Therefore, although IRES are pervasive, the duration and extent of the non-flow period is highly dependent on catchment size, morphology, lithology and climate (Williams, 2006).

In addition, it is expected that climate change and anthropogenic activities, such as water abstraction or damming, will severely affect fluvial biodiversity and ecosystem services through the alteration of flow activity patterns. For instance, while overexploitation of water resources via, for instance, flow regulation, surface and groundwater abstraction or water diversion for irrigation can increase the spatial prevalence of IRES (Vörösmarty & Sahagian, 2000; Datry *et al.*, 2017b), wastewater effluents from urban areas or agricultural water drainage can mean that naturally IRES adopt permanent flows in a process called "perennialization". For example, during the dry phase when water flow naturally ceases, IRES channels can be used to convey water for livestock and irrigation (Chiu *et al.*, 2017).

Hydrology of IRES

IRES hydrology is characterized by the loss of flow and surface water along the fluvial channel. Flow cessation starts with a contraction phase (flow gradually decreases), followed by the drying of riffles and the formation of

isolated pools (fragmentation), to the point where surface water completely vanishes (non-flow phase) **(Figure 2)**. Dry streambeds might or might not be connected by subsurface flow, creating aquatic terrestrial habitat mosaics (Sponseller *et al.*, 2013). In some streams, during flow cessation and the non-flow period, the subsurface zone is particularly important to maintain biodiversity and the functioning of different ecosystem processes (e.g., organic matter decomposition), because it provides the last resort for water-dependent organisms (Arce *et al.*, 2019). In this way, the subsurface zone could act as an active biological hotspot during the non-flow periods (Boulton *et al.*, 1998, 2010b; Marxen *et al.*, 2010; Stubbington, 2012). However, the extent to which the wet subsurface zone can maintain ecosystem functioning and diversity during these non-flow periods remains unquantifiable (this issue is explored in chapter 1). After the non-flow phase, flow resumes (expansion phase, i.e., rewetting) giving birth to a new hydrological cycle **(Figure 3)**. During the expansion phase, the physicochemical and biotic processes resume; for instance, aquatic habitat reconnection, the release of dissolved nutrients and dissolved organic matter from the substrates where they have accumulated on the dry riverbed (e.g., leaves, wood or animal carcasses) and, the activity of aquatic organisms all start again (Shumilova *et al.*, 2019).

The frequency (e.g., number of times per year), timing (season when it occurs) and duration of flow cessation can be crucial for aquatic organisms and biogeochemical processes that rely on flow activity (Larned *et al.*, 2010; Costigan *et al.*, 2016; Leigh *et al.*, 2016). However, the effects that the frequency and intensity of flow intermittence have on biota and ecosystem functioning remain poorly understood.

Figure 2. Photographs of streams sampled for the work presented in this thesis, during different periods of flow intermittence.

Additionally, seasonal variability of flow (flowing and non-flowing cycles) alter hydrological connectivity in one or more spatial dimensions, with repercussions on physical, chemical and biological processes **(Figure 3)**. For instance, longitudinally, flow cessation halts transport of sediments and nutrients downstream and also biota dispersal (Rolls *et al.*, 2012); laterally, the transfer of energy and sediment is interrupted (Paetzold *et al.*, 2006); and vertically, flow between the hyporheic zone and streambed ceases (Boulton *et al.*, 2010b).

Thus far, it is known that the wet-to-dry and dry-to-wet transitions can follow different trajectories with abrupt changes in environmental conditions (Lake, 2003; Foulquier *et al.*, 2015). For instance, non-flowing conditions lead to reduced dissolved oxygen concentrations, and to increased water temperature, nutrients concentrations and conductivity (Krauss *et al.*, 2011). Water availability also affects the quality and quantity of riparian vegetation input to the river bed (Sanpera-Calbet *et al.*, 2017b). Indeed, the wet-to-dry transition may last from days to years, depending on the rainfall pattern and the connection with the subsurface flow. Nevertheless, the dry-to-wet transition occurs rapidly: within minutes or hours, up to days or maybe weeks (Anna *et al.*, 2009). In general, the intensity and frequency of flow

interruption and resumption could shape how aquatic communities, organic matter processing and nutrient transport respond to flow intermittence, ultimately affecting ecosystem functioning.

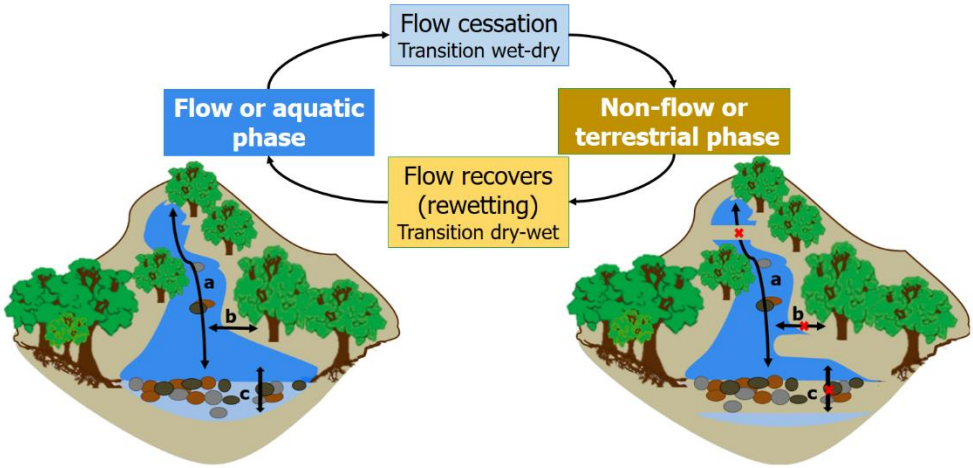


Figure 3. Conceptual diagram representing the hydrological cycle in IRES and spatial hydrological connectivity: longitudinal (a), lateral (b) and vertical (c) connectivity. Red crosses represent loss of hydrological connectivity (Modified from Datry *et al.*, 2017a).

Characterization of flow intermittence

It is currently recognized that the non-flow periods are the main challenge facing aquatic biota and ecosystem functioning in IRES. To characterize biological responses to intermittency, the most common approaches treat flow intermittence as a categorical phenomenon, either in terms of non-flowing phase duration (e.g., permanent *vs.* IRES) or hydrological connectivity (e.g., connected vs. disconnected habitats) (Bogan & Lytle, 2007; Gallart *et al.*, 2017; Stubbington *et al.*, 2018). Such approaches limit the scope of inference along the gradient of intermittency, as has been described by different studies (Poff *et al.*, 1997; Patrick & Yuan,

2017). Various authors have considered five key continuous components of flow activity and variability, to describe flow regimes, and devise meaningful relationships between hydrology and both stream community composition and ecosystem integrity: magnitude, frequency, duration, timing and rate of change.

Nonetheless, an alternative approach has grown over recent years that consider intermittency as a quantitative variable. The characterization of the non-flow period is based, for example, on the mean number of days per year without flow or the proportion of the year without flow (Belmar *et al.*, 2011, 2019; Datry *et al.*, 2014b; Schriever *et al.*, 2015). However, other flow components of IRES hydrology could also be ecologically relevant, such as the timing and intensity of extreme flows after drying, the frequency of non-flow periods, the predictability and duration of non-flow periods or the time and duration of flow recovery (rewetting) (Poff *et al.*, 1997; Patrick & Yuan, 2017; Muñoz *et al.*, 2018).

Despite the global importance and prevalence of IRES, the adequate characterization of their complex flow regimens is not yet consolidated, calling for more precise *in situ* assessment of flow conditions. To remedy these limitations, a novel and low-cost methodology is currently available to characterize stream flow through continuous temperature and/or water level measures. Data-loggers (with software that records sequential data to a log file) are a promising tool capable of continuously quantifying flow intermittence components **(Table 1)** over long periods of time (Patrick & Yuan, 2017).

Table 1. Glossary of the hydrological metrics used to characterize flow intermittence in this thesis.

The data are collected using Leveloggers (Solinst Levelogger Edge; full-scale reading precision, 0.05%) placed on the streambed and from Barologgers (Solinst Barologger; full-scale reading precision, 0.05%) installed at each site, within the riparian area (for more details, see methodology of Chapters 1, 2 and 3).

Hydrological metric	Definition
Total Non-Flow days (TNF)	The sum of the total number of non-flow days
Non Flow Periods (NFP)	Number of non-flow periods
Non Flow (NF)	Number of days in the last non-flow period
Rewetting (RE)	Number of flowing days since the last non-flow period

Ecosystem functioning in IRES: organic matter decomposition

Changes in local biodiversity and abiotic conditions in response to flow intermittence are expected to result in a varying capacity to sustain ecosystem functions such as primary and secondary production, organic matter decomposition or maintaining the food web structure (Vázquez *et al.*, 2007; Abril *et al.*, 2016; Belmar *et al.*, 2019; Soria *et al.*, 2020). These functions are key for biogeochemical cycles of major elements; they transfer energy to higher tropic levels such as predatory fish and birds, and provide crucial services such as supplying clean water or offering recreation (De Crespin De Billy & Usseglio-Polatera, 2002; Butler *et al.*, 2009; Woodward *et al.*, 2012; Green & Elmberg, 2014; Perkins *et al.*, 2015; Schmitz, 2017).

Organic matter (OM) decomposition is a key ecosystem process that has implications for aquatic food webs and global biogeochemical cycles, such as C-cycling pathways (Gessner *et al.*, 1999; Follstad Shah *et al.*, 2017). In forested streams, the main source of OM is riparian vegetation, in the form of leaves or woody debris, and this represents the main basal source for food webs (Vannote *et al.*, 1980; Gonçalves *et al.*, 2014; Abril *et al.*, 2016). Decomposition rates depend on both biotic and abiotic factors (Krauss *et al.*, 2011). Among decomposers, microbes (fungi and bacteria) and invertebrates are the most important organism; but their decomposing activities vary in response to local environmental characteristics (Gessner *et al.*, 2010), such as those produced by flow intermittence.

Once OM enters a stream, the abiotic release (leaching process) of dissolved OM (DOM) accounts for up to 25 % of mass loss over the initial few days (Bärlocher & Sridhar, 2014). After leaching, there is a microbial conditioning phase, which constitutes an essential trophic step for invertebrate consumers, such as shredders, through the increase of OM palatability (Kuehn, 2016). Aquatic hyphomycetes are the main microbial drivers of conditioning, because they are the first colonizers of OM, and they account for more than 95% of the total microbial biomass (Duarte *et al.*, 2010). In fact, flowing water stimulates their sporulation process and supplies OM with a continuous source of fungal spores **(Figure 4)** (Pringle, 2003; Graça *et al.*, 2005; Artigas *et al.*, 2009; Abril *et al.*, 2016; Gonçalves *et al.*, 2016). Once fungi become established on OM, conidia from different taxa germinate and the fungal mycelium increases the nitrogen and phosphorus contents of OM substrates. Moreover, fungal mycelia penetrate and degrade leaf tissue through the action of their extracellular enzymes, transforming recalcitrant polymers into more labile molecules (Romaní *et al.*, 2006). Consequently, aquatic hyphomycetes improve the palatability and nutritional quality of OM

by increasing its protein, lipid and carbohydrate content (Suberkropp *et al.*, 1983; Müller-Navarra *et al.*, 2000; Chung & Suberkropp, 2009; Mora-Gómez *et al.*, 2016; Sanpera-Calbet *et al.*, 2017a). This enrichment process is crucial for shredding invertebrates: it stimulates their OM consumption. Finally, after microbial decomposition, the OM becomes softer and starts to undergo physical abrasion and fragmentation by shredders: the last step of decomposition. This can be responsible for the loss of up to 60% of initial leaf mass (Hieber & Gessner, 2002). Because of physical and biological fragmentation, during this phase, part of the OM is converted into fine particulate organic matter (FPOM) or DOM (Gessner *et al.*, 2010) **(Figure 5)**. Hydrological connectivity allows the continuous colonization of OM by microbial and invertebrate decomposers, but what happens when flow ceases in IRES?

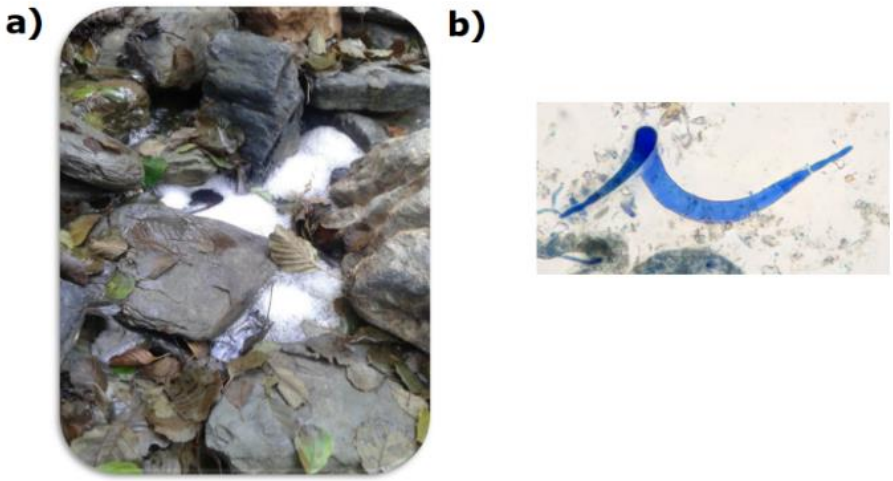


Figure 4. Example of foams in fluvial ecosystems where we can find aquatic hyphomycetes spores (a). An example of conidia of *Anguillospora crassa* (b).

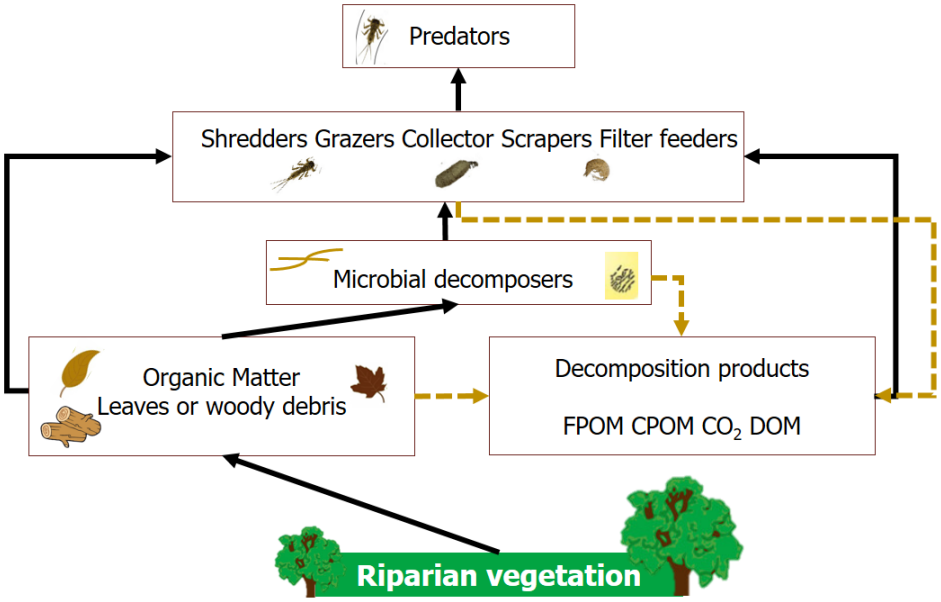


Figure 5. Simplified conceptual scheme of a typical detrital-based food web (flux of energy and material) and the relationships among the trophic levels involved in organic matter decomposition in forest streams. Black arrows represent the relationship among the trophic levels; orange dashed arrows represent decomposition products. CPOM: coarse particulate organic matter, FPOM: fine particulate organic matter, DOM: dissolved organic matter (Modified from Gessner *et al.*, 2010; Trzcinski *et al.*, 2016).

Recent evidence has shown that OM decomposition can be compromised in IRES (Larned *et al.*, 2010; Datry *et al.*, 2011, 2018b; Abril *et al.*, 2016). Studies suggest that flow reduction and cessation may have exert different effects on aquatic decomposers, depending on the length and frequency of non-flow phases and on the availability of refuge for aquatic biota, i.e., aquatic life can persist during non-flow periods in isolated pools or wet sediments (Solagaistua *et al.*, 2016; Burrows *et al.*, 2017). Indeed, it is

known that microorganisms and invertebrate shredders seek refuge by moving vertically down into the subsurface since it is the last remaining habitat where water is available **(Figure 6)** (Stubbington, 2012; Arce *et al.*, 2019). Moreover, flow fragmentation exerts a strong influence on riparian vegetation, causing early leaf abscission (Sanpera-Calbet *et al.*, 2016). Therefore, large amounts of OM that accumulate on dry streambeds may become buried in sediments during later storms (Scott & Zhang, 2012), resulting in the subsurface zone being the most important OM storage compartment in most streams (Storey *et al.*, 1999; Cornut *et al.*, 2012). Water is usually maintained in the subsurface zone, and is a biochemically active zone during the non-flow period (Boulton *et al.*, 1998, 2010b; Marxen *et al.*, 2010; Stubbington, 2012; Gionchetta *et al.*, 2019).

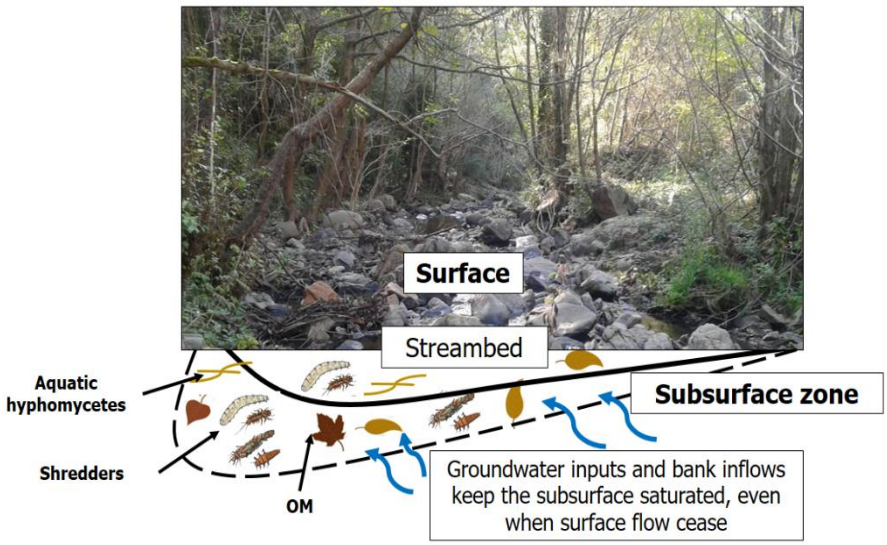


Figure 6. Conceptual scheme of possible activity in the subsurface zone during a non-flow period (Modified from Burrows *et al.*, 2017).

However, it remains unclear whether the subsurface compartment can support rates of OM decomposition that are similar to or higher than those of the wet surface zone when flow is present, and how fungal communities mediate this process (see Chapter 1). Therefore, a comprehensive understanding of OM decomposition in IRES should go beyond the sole consideration of the surface zone and include interactions with the subsurface zone.

In addition, flow intermittence reduces the activity, variety and abundance of both fungal decomposers and invertebrate shredders (Gessner & Chauvet, 1994; Gessner *et al.*, 2010; Bruder *et al.*, 2011; Datry *et al.*, 2011; Martínez *et al.*, 2015; Gonçalves *et al.*, 2016). This, in turn poses a risk for the efficiency of OM decomposition (Lake, 2003; Foulquier *et al.*, 2015). Previous studies have revealed that the growth and development of microbes is highly sensitive to changes in environmental temperature and dissolved nutrient or oxygen contents, and that they are intimately associated with the OM substrate (Chauvet *et al.*, 2016). Indeed, most microbes are particularly vulnerable to harsh environmental conditions resulting from desiccation due to their low mobility (Foulquier *et al.*, 2015). Some previous manipulative studies have shown that simulated flow reductions can have a negative impact on aquatic hyphomycete richness and produce alterations in both taxonomic composition and enzymatic activity (Gonçalves *et al.*, 2016; Mora-Gómez *et al.*, 2016), ultimately affecting leaf litter decomposition. Nevertheless, it is unknown to what degree fungal communities can recover from desiccation **(Figure 7)** and whether manipulative findings apply to real systems, especially across larger spatial scales (see Chapter 3). It is known that aquatic hyphomycetes show adaptations to flowing waters, including conidia shape (mostly tetracladiate, branched or elongated), the production

of mucilage and rapid germination (Read *et al.*, 1992), but it is not clear if they present adaptations to non-flow periods (Chapter 3).

Moreover, flow intermittence may produce bottom up changes in the trophic chain due to alterations in OM quality resulting from desiccation (Sanpera-Calbet *et al.*, 2017b). Invertebrate shredders may tend to select the most energetic and nutrient-rich food available, to enhance their fitness (Mas-Martí *et al.*, 2015, 2017); but OM palatability could depend on fungal activity, which could in turn be modified by intermittency. Leaf litter dynamics (e.g., accumulation and distribution) during the dry rewetting cycle in the riverbed could also determine food availability for microbes and invertebrates. So, the quality and quantity of OM is affected by desiccation, but little is known regarding the effect of these combined changes on the feeding preferences and growth of invertebrate shredders (see Chapter 4).

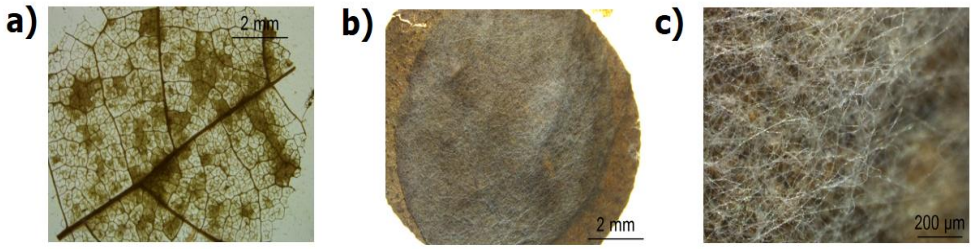


Figure 7. Example of: alder leaf disc after two weeks of decomposition (a); and aquatic hyphomycete mycelia after two weeks of non-flow (b and c).

Aquatic communities in IRES

IRES are complex, dynamic and diverse ecosystems in which the shifting habitat mosaics associated with intermittency drive the composition, abundance and diversity of aquatic communities (Datry *et al.*, 2016). Their seasonal variability in flowing water acts as an environmental filter for varying degrees of organism tolerance to environmental conditions resulting from flow cessation (Boulton, 2003) **(Figure 8)**.

Environmental filtering is one important process that determines which part of a regional species pool can persist in a specific local environment, thanks to their functional traits or phenotypes (Poff, 1997; Datry *et al.*, 2014b; Gutiérrez-Cánovas *et al.*, 2019). As a consequence, the available evidence indicates that non-flow periods lead to severe changes in the taxonomic diversity and composition of aquatic communities (Muñoz & Prat, 1994; Arscott *et al.*, 2010; Datry, 2012; Bruno *et al.*, 2019). For example, most studies focus on invertebrate taxonomic diversity where they indicate that alpha diversity, i.e., richness (defined in **Table 2** below), declines with increasing flow intermittence (Soria *et al.*, 2017) while spatial beta diversity (defined in **Table 2** below) increases with intermittence (Schriever & Lytle, 2016). The decline in alpha diversity reflects the loss of taxa at critical thresholds due to the reduction of water flow, changes in water physicochemical characteristics and the reduced quality and quantity of food resources, i.e., OM availability **(Figure 8).** The increase in beta diversity reflects habitat heterogeneity related to flow fragmentation (e.g., combinations of pools, riffles or the dry riverbed).

Functional traits (defined in **Table 2** below) can help in predict responses to flow intermittence, but they also provide better understanding of the

functional consequences of biodiversity changes (Diaz *et al.*, 2008; Díaz *et al.*, 2016; Aspin *et al.*, 2019), mainly via functional redundancy (defined in **Table 2** below) among taxa, i.e., different taxa perform similar functions but have contrasting sensitivity to intermittency (Boersma *et al.*, 2014). This redundancy seems to reflect the prevalence of taxa with traits that promote resistance and resilience to disturbance (Vorste *et al.*, 2016). Aquatic organisms employ a wide range of strategies to survive, including the use of a variety of resistance mechanisms to withstand disturbance *in situ* and resilience mechanisms to recover following the disturbance (Lake, 2000; Nimmo *et al.*, 2015). For example, many invertebrate species present desiccation-resistant dormant stages during non-flow periods and re-emerge as active individuals when flow returns (Williams, 2006); or some organisms resist the depletion of dissolved oxygen in water in isolated pools when flow vanishes through the use of aerial respiratory structures (Aspin *et al.*, 2019). In contrast, resilience mechanisms facilitate recolonization of dried reaches from permanent reaches or other streams. For instance, many invertebrate adult stages have the capability to fly, so adults from perennial streams can travel to IRES when flow returns (Datry *et al.*, 2016). Although significant advance have been made in understanding resistance and resilience processes in IRES, many questions are still open, especially concerning aquatic organisms other than invertebrates: fungal species, for example, even though they are a group that is essential to sustaining key ecosystem functions (see section above) (Chapter 3). Thus, one major challenge to forging links between flow intermittence and biotic responses is the adequate characterization of the different faces of aquatic biodiversity (Hurtado *et al.*, 2019), including taxonomic diversity (TD) or functional diversity (FD) (defined in **Table 2,** below) (Chapters 2 and 3).

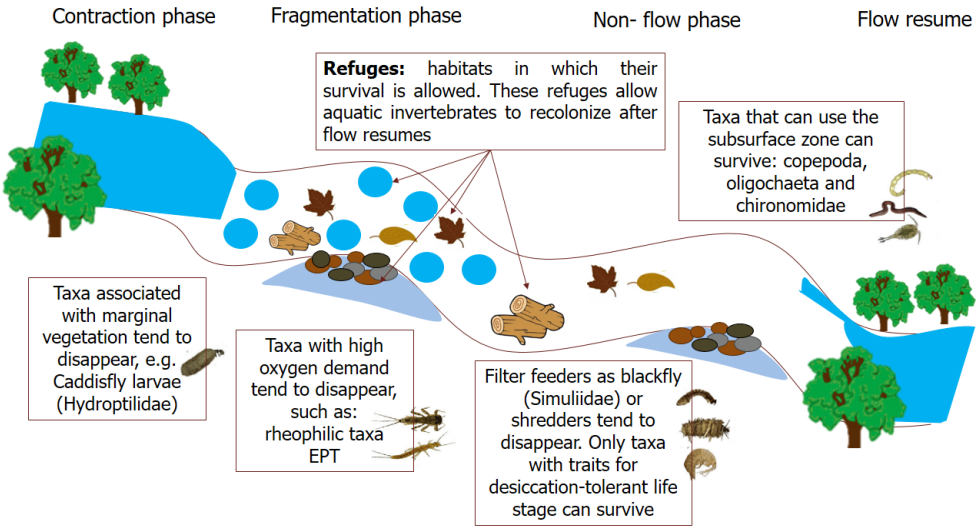


Figure 8. Example of how flow intermittence reduces invertebrate richness. E=Ephemeroptera, P= Plecoptera and T= Tricoptera. Modified from Chapter 4, Section 3 (Datry *et al.*, 2017a).

Current conceptualizations of the effects of flow intermittence on aquatic biota focus on the total number of non-flowing days, while other hydrological aspects that define the window for organism colonization, growth or reproduction can also affect aquatic communities. For instance, Lake (Lake, 2000) and Lepori & Hjerdt (Lepori & Hjerdt, 2006) emphasize the role of the hydrological regime as the most important driver for temporal variation in species diversity. Meanwhile, Larned et al., (Larned *et al.*, 2010) focus on the role of aquatic habitat heterogeneity to explain taxon number and composition. The role of temporal scaling in the structuring of biological communities in IRES remains elusive, which limits our capacity to predict ecosystem trajectories in response to global change (see Chapter 2).

Regarding temporal scales, annual and recent disturbance dynamics act as two sequential hydrological environmental filters, i.e., the disturbance

regime (hereafter termed intermittence regime) and the disturbance status (hereafter termed intermittence status), which can influence the type of organisms that can thrive in IRES (Tonkin *et al.*, 2017; Rolls *et al.*, 2018). First, the intermittence regime is predictable, and determines yearly recurrence of events in which abiotic conditions allow organisms to colonize and develop in a given habitat (e.g., total number of non-flow days per year). This filter selects those organisms that can complete their life cycle under such conditions, distinguishing between tolerant and intolerant organisms (Lytle & Poff, 2004; Bruno *et al.*, 2016a). Second, the intermittence status varies seasonally and provides information on recent disturbance events that determine the moment when habitats can be colonized and the window for growth and reproduction (e.g., days since the last non-flow period, i.e., rewetting). This seasonal filter determines when well-adapted, tolerant organisms can colonize a given habitat and the time they have to grow and reproduce (McMullen *et al.*, 2017). However, despite its potential implications, the role of temporal scaling in structuring biological communities in IRES remains elusive, limiting our capacity to predict their ecosystem trajectories in response to global change (Chapter 2).

Alongside this incomplete characterization of flow intermittence, stream communities have only partially been described, especially microbes, generally, in relation to trait-based metrics. Studies have focused on taxonomic aspects of invertebrates that consider species as functionally equivalent (e.g., taxon richness or abundance; TD), this, does not allow their mechanistic relationship with environmental filters and ecosystem functioning to be inferred (Hooper *et al.*, 2005). Hence, the use of FD based on trait measurements can be more helpful in explaining invertebrate responses to the different hydrological components of flow intermittence. FD is defined as the diversity of functional characteristics within an ecological

community based on morphological, behavioral or life-history traits, and it is related to ecosystem functioning and stability (Villéger *et al.*, 2008; Mouillot *et al.*, 2013). Many studies have demonstrated that FD, such as functional richness, functional dispersion and functional redundancy, account for different natural and anthropogenic impacts on aquatic or riparian communities (Gutiérrez-Cánovas *et al.*, 2015; Bruno *et al.*, 2016b; Soria *et al.*, 2020). Hence, a better understanding of FD responses to flow intermittence components has the potential to improve our capacity to manage and preserve IRES. Moreover, including the use of response-effect trait framework (Suding *et al.*, 2008) offers a good opportunity to reveal these links (Suding *et al.*, 2008; Laliberté *et al.*, 2010; Lavorel *et al.*, 2013; Palmer & Ruhi, 2019). This framework distinguish morphological, behavioral and life-history traits that enable organisms to cope with the hydrological regime (response traits) and other aspects (e.g., functional feeding strategies) that support ecosystem functioning (effect traits) through trophic relationships **(Figure 9)**. Despite recent progress, it remains unclear how the different components of flow intermittence will affect different aspects of aquatic biodiversity, including both taxonomic and trait-based metrics (Chapter 2).

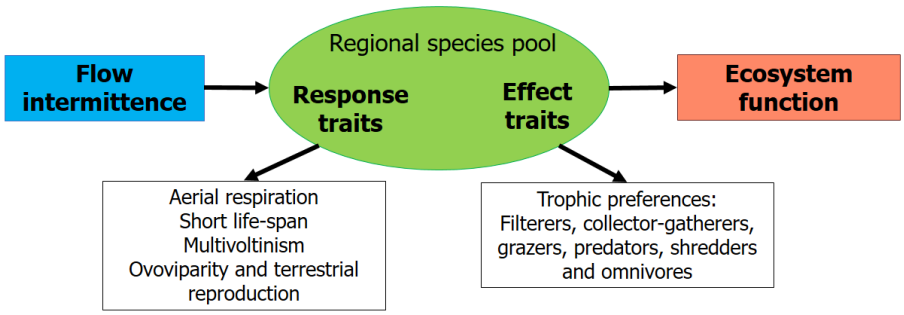
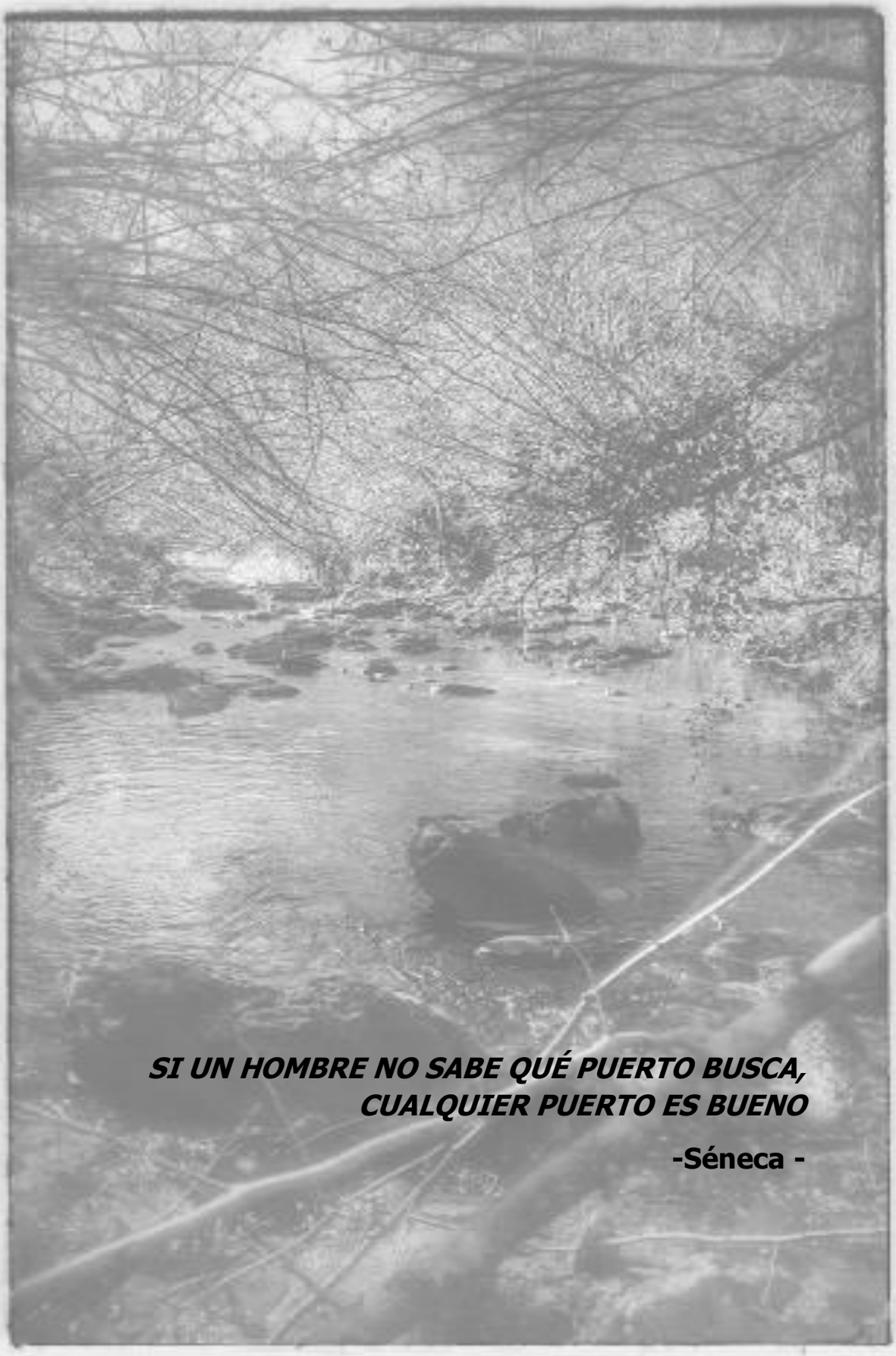


Figure 9. Conceptual response-effect trait framework including the response and effect traits used in this thesis.

Table 2. Glossary of diversity metrics used in this thesis (based on Mouillot *et al.*, 2013; Rolls *et al.*, 2018).

Taxonomic diversity (TD)	The variety and range of species based on taxonomic measures at different spatial scales.
Trait	Any morphological, physiological, or phenological feature usually measured at the individual level.
Functional trait	Any trait that influences organism performance (either as a response to environmental changes, i.e., response traits such as a short life-span or aerial respiration, or as a measure of individual contribution to ecosystem functioning, i.e., effect traits such as trophic groups).
Functional space	A multidimensional space where the axes are functional traits (either response or effect traits) along which species are placed according to their functional trait value.
Functional diversity (FD)	The distribution and range of species and their abundance in the functional space of a given community.
Alpha diversity	The local diversity within a community present at a particular site or habitat, and it refers to the number of taxa (TD) or functional diversity (FD) at a location.
Beta diversity	Spatial variation in the composition of taxa or traits between locations. Beta diversity has two components: turnover (replacement of species or traits) and nestedness-resultant dissimilarities (nested subsets of species or traits) (Baselga, 2010).
Functional richness	The volume of functional space occupied by all the species within a community.
Functional dispersion	The proportion of total abundance supported by species with the most extreme trait values within a community.
Functional redundancy	A measure of the overall representation of the different functional groups, typically estimated as the total abundance divided by the number of functional groups.
Response or effect-trait diversity	Abundance-weighted functional dispersion based on response or effect traits and the individuals belonging to that group (Elmqvist *et al.*, 2003).

RESEARCH OBJECTIVES



SI UN HOMBRE NO SABE QUÉ PUERTO BUSCA,
CUALQUIER PUERTO ES BUENO

-Séneca -

RESEARCH OBJECTIVES

Using a combination of manipulative and observational approaches, this thesis focuses on the effects of flow intermittence on biodiversity and ecosystem functioning in Mediterranean streams. Specifically, I focus on how flow intermittence affects organic matter decomposition and aquatic communities (fungi and invertebrates) over a continuous flow intermittence gradient, which was measured *in situ* and ranging from permanent rivers to ephemeral streams. Aquatic fungi are the main microbial drivers of OM decomposition in streams and they constitute an essential link with higher trophic levels. Meanwhile, invertebrates are a diverse multi-trophic representation of a broader aquatic community. The main body of this thesis is divided into four chapters; each one written as an independent publication **(Figure 10)**.

In Chapter 1, I explore **the dynamics of microbial decomposition in the surface and subsurface zones** in 20 streams over a gradient of flow intermittence. First, I explore **the effects of hydrology and micro-habitat (surface and subsurface zone) on organic matter decomposition and fungal biomass**, and then I analyze the effects of hydrological and **environmental features** on organic matter decomposition and fungal biomass in each zone separately. I hypothesize that: (i) the rates of organic matter decomposition and the fungal biomass will be higher in the subsurface zone than in the surface compartment when the intermittency increases, which will sustain the processing of organic matter, and (ii) environmental features will modulate hydrological effects on organic matter decomposition through changes in the microbial communities (e.g., fungal biomass).

In Chapter 2, using invertebrate communities from 33 streams across a broad flow-intermittence gradient, I assess **the effect of the annual regime and recent status of intermittence disturbance on biodiversity components,** including both taxonomic and functional-trait-based metrics, i.e., based on either response or effect traits. I also assess **the effect of widely used non-hydrological (environmental) filters** (climate, geomorphology, land-use and water chemistry). To do that, I first characterize stream hydrology in depth, over one year before sampling, using metrics that describe the intermittence regime (total non-flow days and number of non-flow events) and intermittence status (duration of the last non-flow period and time since rewetting). After that, I assess how these intermittence filters (i.e., regime and status), together with non-hydrological filters, influence whole community taxonomic and trait-based metrics, i.e., richness, Shannon diversity, total abundance, functional diversity, and functional redundancy. Finally, I explore how trophic groups respond to hydrological and non-hydrological filters by studying changes in their abundances and response trait diversity. I hypothesize that hydrological filters will account for more taxonomical and functional variation than non-hydrological filters. In addition, I hope to determine which aspect of flow intermittence is most important for structuring aquatic life in IRES and could therefore help to monitor these highly dynamic water courses better.

In Chapter 3, using an observational field study, including 15 streams and a manipulative microcosm experiment, I test **the links between aquatic hyphomycete communities and organic matter decomposition**. First, based on observational data, I analyze **how aquatic hyphomycete richness and composition** (overall beta diversity as well as its turnover and nestedness components) **are affected by flow intermittence and how these community changes affect organic matter**

decomposition. Secondly, I explore **how leaf litter decomposition responds to the joint effects of fungal richness and flow intermittence** in a microcosm setting. I use two manipulated levels (low and high) of aquatic hyphomycete richness and three treatments simulating increasing flow intermittence (permanent, intermittent and ephemeral flow regimes) to analyze their effects on leaf litter decomposition. In addition, in the intermittent treatment, during rewetting, I analyze how aquatic hyphomycetes recover from the non-flow period. Firstly, I hypothesize that flow intermittence will reduce aquatic hyphomycete richness; and secondly, that the persistence of aquatic hyphomycete communities is primarily associated with resistance mechanisms, i.e., by community turnover processes across the flow intermittence gradient. Finally, I expect richness to mediate the effect of flow intermittence on organic matter decomposition.

In Chapter 4, using field and microcosm approaches, I explore **how changes in both the leaf litter quality and quantity affect or determine the feeding preferences and growth of an invertebrate shredder**. First, I assess the influence of flow intermittence on leaf litter quality (fungal biomass, C:N ratios and total lipid content) and on the composition of the associated community of aquatic hyphomycetes. I expect that under flowing conditions (a permanent stream) leaf litter will be of better quality than that from an intermittent stream. Second, I explore the joint effects of leaf litter (a food resource) quality and quantity on the consumption and growth rates of a shredder, using microcosms. I expect that better quality food resources and availability will correlate with higher consumption and growth rates of the shredder. Finally, I quantify the feeding preferences of the shredder, with the expectation that the quality rather the quantity of the resource will be more important. Therefore, even if the

shredder has a larger quantity of poor-quality resources, it will actively select the best quality food.

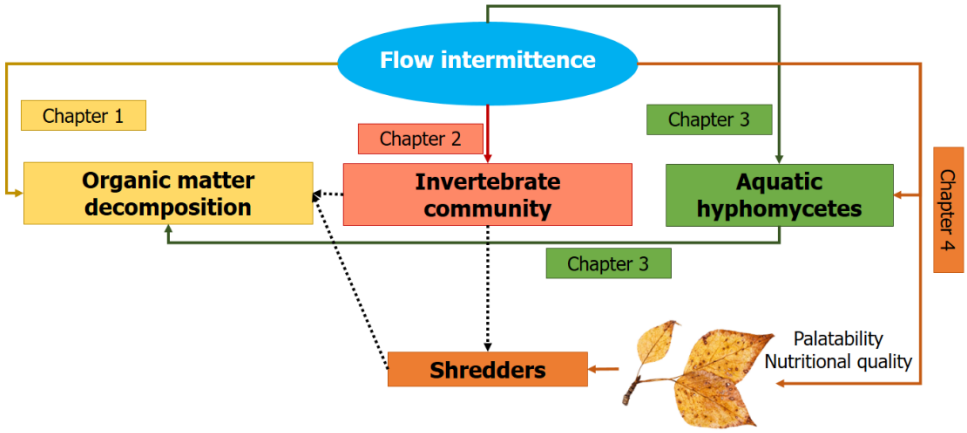
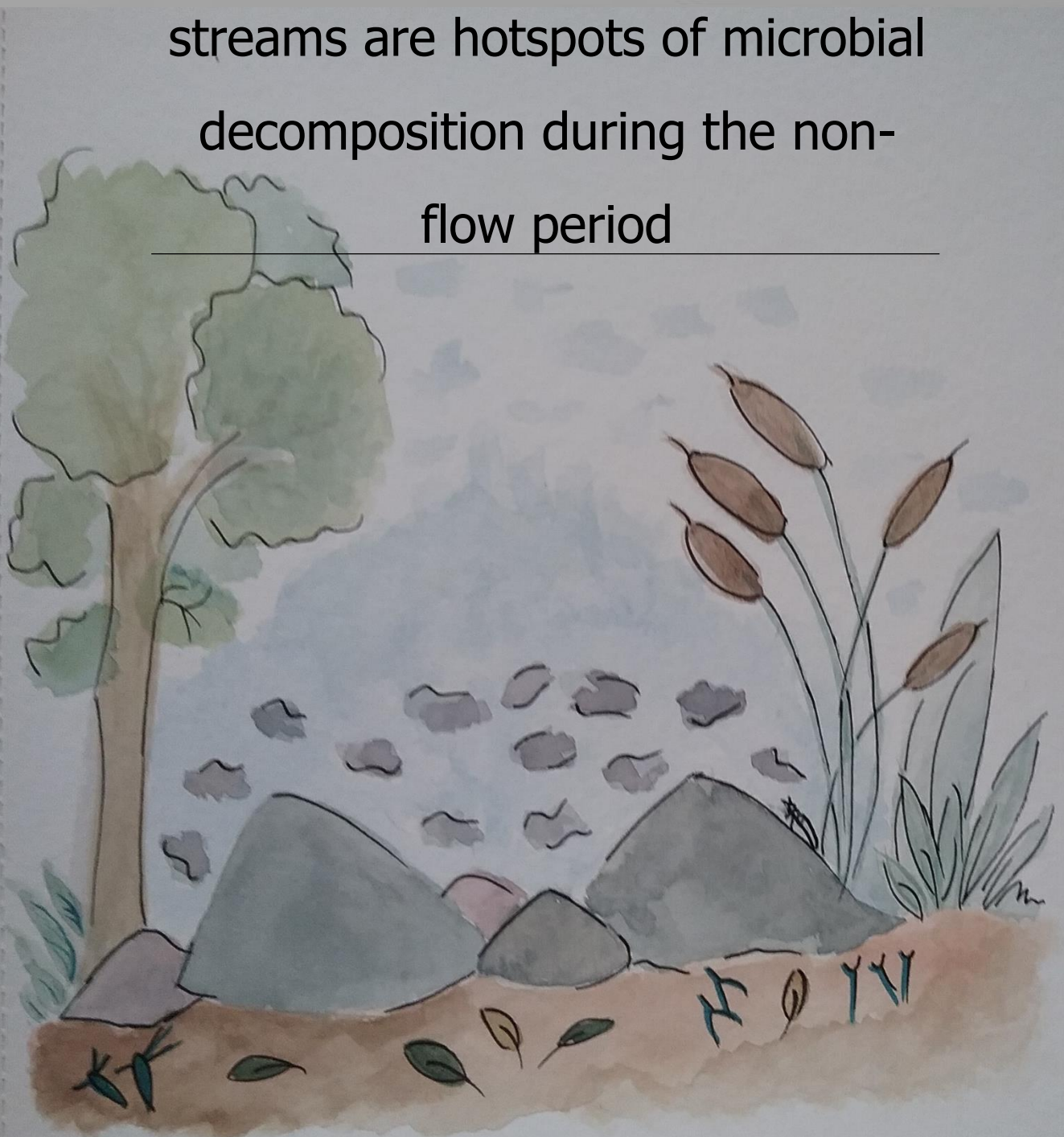


Figure 10. Scheme of the chapters in this thesis. Broken arrows represent indirect results of this research.

CHAPTER 1

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



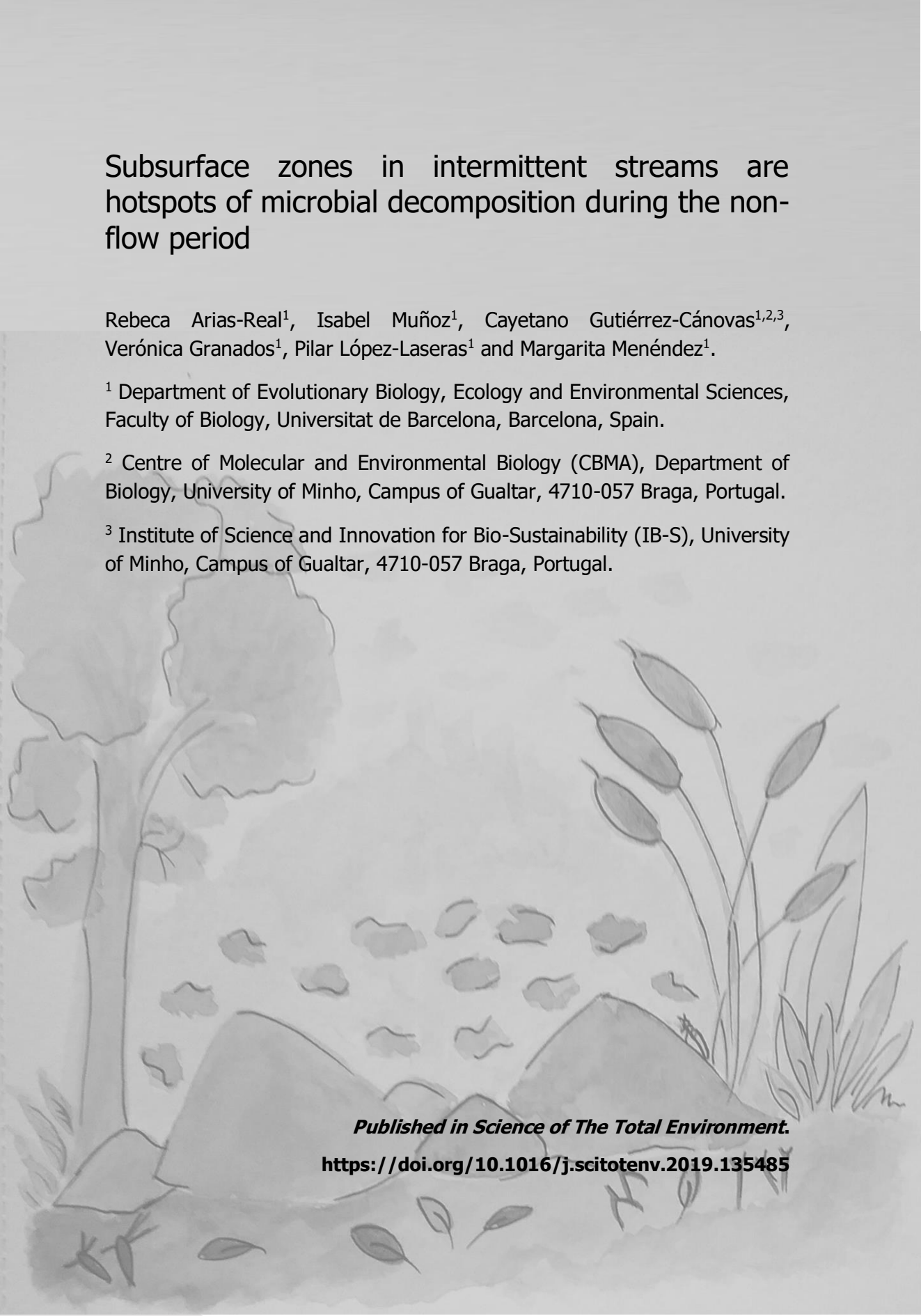
Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period

Rebeca Arias-Real[1], Isabel Muñoz[1], Cayetano Gutiérrez-Cánovas[1,2,3], Verónica Granados[1], Pilar López-Laseras[1] and Margarita Menéndez[1].

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Barcelona, Spain.

[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

[3] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.



***Published in Science of The Total Environment*.**

https://doi.org/10.1016/j.scitotenv.2019.135485

Abstract

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

Introduction

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

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

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

However, flow intermittence may exert different effects on aquatic decomposers depending on the length and frequency of non-flow phases and the characteristics of different stream microhabitats (Solagaistua *et al.*, 2016; Burrows *et al.*, 2017). For example, aquatic life can persist during the non-flow phase in isolated pools, wet sediments and the hyporheic zone, but

the suitability of these microhabitats could vary with grain size, solar irradiance or weather (Marxen *et al.*, 2010; Datry *et al.*, 2011; Stubbington, 2012; Pohlon *et al.*, 2013; Harjung *et al.*, 2019). In addition, some flash storms can rapidly stimulate and restore microbial activity (Blazewicz *et al.*, 2014; Barnard *et al.*, 2015; Gionchetta *et al.*, 2019). Therefore, considering that IRES represent approximately half of the global river network and that their spatial extent is expected to increase due to climate change and increased water use (Datry *et al.*, 2017b), there is an urgent need to better understand which hydrological, microhabitat and local environmental factors can sustain OM decomposition during the non-flow period.

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

Materials and methods

Study area

This study was conducted in 20 low-order streams that belong to eight different basins across Catalonia (NE Spain) **(Figure 11 and S1-Figure 1)**. Forest, scrubland and grasslands were the primary land use at the riparian scale **(Table 3)**. Although, in some streams, the main land use was extensive agriculture (mainly olive groves and vineyards), causing minor levels of anthropogenic impact (Corine Land Cover 2006 data from a buffer area of 1 km around each sampling site) (Table 1). Furthermore, poplar *(Populus nigra* L.), alder (*Alnus glutinosa* (L.) Gaertner) and evergreen oak (*Quercus ilex* L.O) were the dominant riparian vegetation. The climate is typically Mediterranean with dry and warm summers, and precipitation occurring mainly during spring and autumn.

Figure 11. Example of sampling sites.

Table 3. Geographical and basin characterisation of the studied sites. The percentages of land use cover refer to a buffer area of 1 km around each sampling point.

Site	Alt.	Ord.	Basin	Prec. (mm)	Catchment area (km2)	Land Use Cover (%)		
						Urban	Agric.	Nat.
1	430	3	BE	788	6.5	2.2	0.8	97.1
2	484	4	FL	1026	15.7	0	1.1	49.3
3	219	4	FL	998	13.8	0	34.4	65.6
4	475	4	FL	475	965.4	0.1	2.8	97.7
5	434	3	FL	963	6.7	0	0	100
6	330	4	FO	590	26.9	0	100	0
7	390	3	FO	574	1.8	0	96.4	3.6
8	605	4	FR	470	28.9	0	14.1	85.9
9	325	3	LL	732	10.9	2.2	0.9	96.9
10	394	3	LL	692	8.6	0	0	100
11	335	4	LL	635	23.9	1.2	2.9	95.9
12	100	3	MU	842	13.5	0	99.5	0.5
13	526	3	TE	963	13.3	0	0.6	99.4
14	630	4	TE	953	30.9	1.4	0	98.5
15	140	3	TE	816	7.7	0	78.5	21.5
16	385	3	TE	1038	9.1	0	0.9	99.1
17	110	3	TO	865	49.1	0	0.6	99.4
18	489	3	TO	911	12.4	0	0.2	99.8
19	655	4	TO	875	49.0	0	0	100
20	170	3	TO	908	12.4	0.8	3.3	95.9

Alt. = altitude (m.a.s.l); Ord. = order; Prec. = precipitation; Agric. = agriculture; Nat. = nature; BE= Besós; FL= Fluviá; FO= Foix; FR= Francolí; LL= Llobregat; MU= Muga; TE= Ter and TO= Tordera.

Stream hydrology

We calculated the total number of non-flow days (TNF) at each site **(Figure 12)**.

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

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

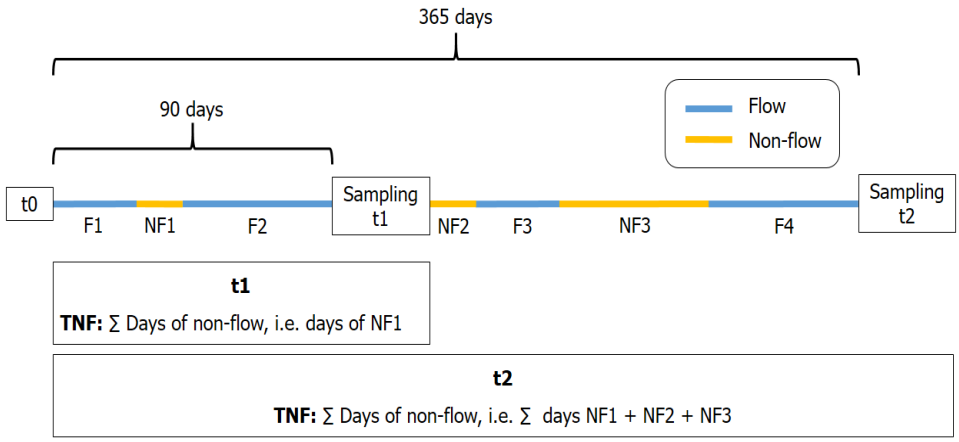


Figure 12. Example of the calculation of total non-flow days (TNF) in this study. t0 is the time when the experiment started and the wood-sticks and loggers were placed on the streams, t1 is the time when we took the first wood-sticks and t2 is the time when we took the remaining wood-sticks and the experiment finished. NF is non-flow, F is flow and TNF is total non-flow days.

Environmental factors

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

To characterise the nutrient concentrations in the water (nitrite, nitrate, ammonium and soluble reactive phosphorus, SRP), we took water samples

when surface water was present. Water samples were filtered through pre-combusted glass fibre filters in the field (0.7 µm pore size; Whatman GF/F, Germany) and then transported to the laboratory under cooled conditions. In the laboratory, we stored the water samples at 4 ºC, in darkness, until analysis (between 24 and 48 hours).

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

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

To determine the grain size distribution, fresh sediment samples (first aliquot) were first treated with H_2O_2 (10% volume) to remove organic matter and later disaggregated and dispersed ultrasonically with pyrophosphate. Fractions up to 2 mm were determined by sieving, while the determination of fractions below 2 mm was performed with a Beckman-Coulter LS230 laser. Then, the dry material (second aliquot) was ground using an agate mortar until it was completely homogenised, and we analysed the nitrogen (N) and

carbon (C) concentrations using a Thermo Elemental Analyser 1108 (Thermo Scientific, Milan, Italy). We expressed the results in terms of C:N molar ratios.

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

Organic matter experiment

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

The sticks that were placed in the streams were picked up during the two sampling campaigns: one after between 90 and 100 days (t1) and the second after one year (t2). During each sampling, we collected five sticks per zone (half of the sticks). The sticks were placed in individual zip-lock bags and transported to the laboratory in refrigerated containers.

Once in the laboratory, we processed the sticks immediately to avoid changes in weight and ergosterol degradation. First, we gently brushed them to remove adhering material and then washed them with distilled water. Afterwards, we cut and weighed one 1-cm-long aliquot of each stick. These aliquots were frozen at -80 ºC for later determination of the ergosterol concentration as a proxy for fungal biomass (Gessner, 2020). Then, the remaining part of each stick was dried (70 ºC, 72 h) and weighed to calculate the final dry mass.

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

To analyse the ergosterol concentration as a proxy of fungal biomass (Gessner, 2020), an aliquot of each stick was lyophilized and weighed to determine the dry mass, and lipid extraction and saponification were performed using 0.14 M KOH methanol (8 g L^{-1}) at 80 °C for 30 min in a shaking water bath. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak® Vac RC, 500 mg tC18 cartridges, Waters Corp, Milford, MA, USA), and ergosterol was eluted using isopropanol. We used high-pressure liquid chromatography (HPLC) to detect and quantify the ergosterol by measuring the absorbance at 282 nm. We used a Jasco HPLC system (USA) equipped with a Gemini-NX 5 µm C18 250 × 4.6 mm column (Phenomenex, UK). The mobile phase was 100% methanol, and the flow rate was set to 1.2 ml min^{-1}. Finally, we converted the ergosterol measurement into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium (Gessner & Chauvet, 1993). We expressed the results in mg of fungal biomass per gram of dry mass.

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

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

Data analysis

To reduce distribution skewness, water sediment, coarse sand, DIN and SRP were log-transformed and clay, fungal biomass and C:N ratios of sediments were square-root-transformed, before the analyses were performed. All quantitative predictors were Z-standardised (mean=0, SD=1) to allow for model coefficient comparison. To assess predictor collinearity, we estimated the Variance Inflation Factor (VIF; vifstep, usdm R package; Zuur *et al.*, 2009, 2010) and pairwise Pearson correlations (cut-off of $r \leq |0.70|$) (Feld *et al.*, 2016). To analyse how OM decomposition (decay rates) and fungal biomass respond to flow intermittence at surface and subsurface zones, we used linear mixed-effect models (LMMs, lme4 R package; Pinheiro *et al.*, 2017). For both response variables, we created

LMMs that included TNF, zone (two-level qualitative predictor: surface and subsurface) and their interaction as fixed factors. These models included data from 20 sites, in which both surface and subsurface zones were surveyed on two occasions (decay rates: n=70; fungal biomass: n=72). Using Akaike Information Criteria (AIC) values, we checked two random structures ("sampling site" and "sampling site nested within basins") to account for non-independent structures within samples belonging to the same sites and basins. We selected "sampling site" as the random structure for both decay rates and fungal biomass models, as this model structure showed a better explanatory capacity and model simplicity (lower AIC values). Furthermore, we used a quadratic term for TNF to account for nonlinear responses. For each LMM, we estimated the variance explained by the fixed factors alone (r^2_m) and the variance explained by both the fixed and the random terms (r^2_c). To explore the relative importance of TNF, zone (surface vs. subsurface) and their interactions in the models, we performed variance partitioning on LMMs using the *variancePartition* R package (Hoffman & Schadt, 2016).

To identify how environmental features modulate hydrological effects on OM decomposition and fungal biomass in surface and subsurface zones, we followed a two-step modelling procedure that included an exploratory analysis to select the most important predictors and final models to estimate environmental features´ importance and significance (Feld *et al.*, 2016). These models included 20 sites surveyed on two occasions (surface zone: n=38 and subsurface zone: n=36).To rank and select predictors according to their predictive power, we used Spearman rank correlations to account for potential non-linear responses. Second, to quantify the effects, importance and significance of hydrology (TNF) and the best environmental predictors of OM decomposition (decay rates) and fungal biomass, we fitted linear

regression models (LMs) and LMMs. Then, between these models, we selected linear mixed model (LMM) for decay rates on the surface zone and linear regression models (LMs) for decay rates on the subsurface zone and fungal biomass in both, surface and subsurface zones, due to their greater explanatory capacity and parsimony compared to LMMs (i.e., lower AIC values) (Akaike, 1973).

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

In the subsurface zone, we used TNF, fine sand, water sediment content and C:N ratios of the sediment as predictors for decay rates in each LM, whereas we used TNF, coarse sand, water sediment content and C:N ratios of the sediment as predictors for fungal biomass in each LM. None of the final input variables included in the final models has a collinearity problem **(S1-Tables 1, 2 and 3)**.

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

All models were validated by visually checking their residuals for normality and homoscedasticity (Zuur *et al.*, 2010). All statistical analyses were performed using the R statistical software version 3.4.1 with the significance level set at $p < 0.05$ for all tests (R Development Core Team, 2011).

Results

The studied streams covered a steep gradient of intermittency (from permanent to ephemeral streams) **(S1-Table 4)**. Dissolved oxygen varied from 4.9 mg L^{-1} to 9.2 mg L^{-1}, conductivity varied from 164.6 µS cm^{-1} to 827.0 µS cm^{-1}, DIN (nitrite + nitrate + ammonia) varied from 0.424 mg L^{-1} to 6.174 mg L^{-1} and SRP varied from 0.008 mg L^{-1} to 1.727 mg L^{-1} in surface flowing water **(S1-Table 5)**. Moisture content varied from 2% to 84%; sediment grain size proportions varied from 0% to 19.31% clay, 0% to 48.21% silt, 0% to 32.98% fine sand, 9.72% to 98.75% coarse sand, and 0% to 61.95% gravel; and the ratios of C:N in the sediments varied from 9.1 to 186.8 **(S1-Table 6).**

Effects of hydrology and zone on OM decomposition and fungal biomass

OM decomposition (decay rates, k dd^{-1}) and fungal biomass were greater in the subsurface zone compared to the surface zone when TNF increased **(Table 4, Figure 13)**, as reflected by the significant interactions between non-flow days and zone **(Table 4)**.

The decay rates at the surface decreased with TNF more sharply than the subsurface decay rates, which even recovered at the most ephemeral sites (TNF > 100 days). Thus, in streams with less than 75 days of non-flow, decay rates (k, dd^{-1}) were higher in the surface zone (Mean ± SE; 0.0031±0.0002) than in the subsurface zone (0.0025±0.0002). However, for streams experiencing more than 75 days of non-flow, decay rates were higher in the subsurface zone (0.0018±0.0003) than in the surface zone (0.0014±0.0002) **(Figure 13a).**

For streams with less than 75 days of non-flow, the contribution of decay rates (relative to the total decay rates, i.e., the sum of the decay rates in the surface and subsurface zones) in the surface zone was 10.23% higher than that in the subsurface zone. For streams with more than 75 days of non-flow, the contribution of decay rates in the subsurface zone was 9.77% higher than that in the surface zone **(Figure 14a).** Furthermore, as we observed in **Figure 14a**, for the streams with more than 75 days of non-flow, the contribution of decay rates in the subsurface zone was only 0.32% less than the contribution of decay rates in the surface zone for the streams with less than 75 days of non-flow. We showed that in the surface zone, fungal biomass hardly changes along the gradient of intermittency; nevertheless, in the subsurface zone, we showed that when the intermittency increases, the fungal biomass increases **(Figure 13b).**

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

For streams with less than 90 days of non-flow, in the surface zone, the contribution of fungal biomass (relative to the total fungal biomass, i.e., the sum of the fungal biomass in the surface and subsurface zones) was 43.2% higher than that in the subsurface zone. For streams with more than 90 days of non-flow, the fungal biomass in the subsurface zone was 19.6% higher than that in the surface zone **(Figure 14b).** Furthermore, as we observed in **Figure 14b**, for the streams with more than 90 days of non-flow, the contribution of fungal biomass in the subsurface zone was only 11.8% less

than the ratio of fungal biomass in the surface zone for the streams with less than 90 days of non-flow.

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

Table 4. Results of the LMMs relating decay rates, k (dd-1, n=70) and fungal biomass (mg FB g DM-1, n=72) to TNF and zone and their interactions. Standardized effect size (SES), standard error (SE), significance and variance explained are shown. Significant variables are highlighted in bold. r^2m: variance explained by the fixed factor alone; r^2c: variance accounting for both fixed and random terms. The quadratic term (2) means that the response is nonlinear.

	Hydrological variables	SES	SE	p-value	Explained variance	r^2_m	r^2_c
OM decay rate, k (dd^{-1})	Intercept	0.01	0.00	<0.001		44.9	73.6
	TNF	-0.00	0.00	**<0.001**	38.4		
	Zone	0.00	0.00	0.121	0.5		
	TNF x zone	-0.00	0.00	**0.002**	3.9		
	TNF^2	-0.00	0.00	**0.032**	1.8		
Fungal Biomass (mg FB g DM^{-1})	Intercept	1.63	0.34	<0.001		26.8	65.1
	TNF	1.19	0.21	**<0.001**	2		
	Zone	0.69	0.23	**0.004**	4.2		
	TNF x zone	-1.13	0.23	**<0.001**	21.1		
	TNF^2	0.88	0.23	**<0.001**	11.1		

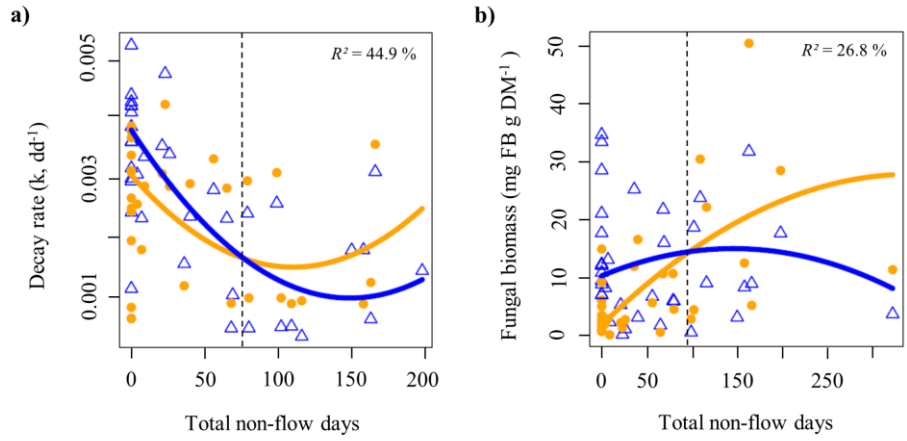


Figure 13. Responses of decay rates (a) and fungal biomass (b) to TNF and their interaction with zone. Fitted lines are shown for the surface (blue) and subsurface (orange) zones in response to non-flow days. Vertical black bars show the temporal point where OM decomposition and fungal biomass in the subsurface zone become greater than the corresponding values at the surface.

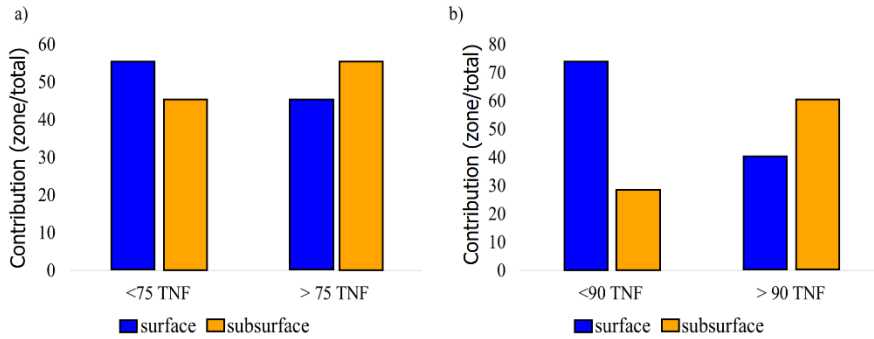


Figure 14. Contribution of decay rates (a) and fungal biomass (b) relative to the total decay rates and fungal biomass, respectively (i.e., the sums of decay rates and fungal biomass in the surface and subsurface zones).

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

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

In the surface zone, decay rates decreased when TNF increased but no other environmental predictor showed a significant effect **(Table 5; Figure 15a)**. In the subsurface zone, decay rates decreased when TNF increased, but the higher presence of fine sand was associated more with higher decay rates and higher moisture content than were lower decay rates with higher water loss (**Table 5, Figures 15b and 15c**, respectively).

Fungal biomass in the surface zone decreased as TNF increased, but higher SRP was linked with higher fungal biomass **(Figure 15d)**. However, in the subsurface zone, fungal biomass increased as TNF increased, and the magnitude of this increase was related to sediment grain size; in the sites with a higher presence of coarse sand, the fungal biomass was lower **(Figure 15e)**. Furthermore, higher C:N content in the sediment was associated with lower fungal biomass **(Figure 15f)**.

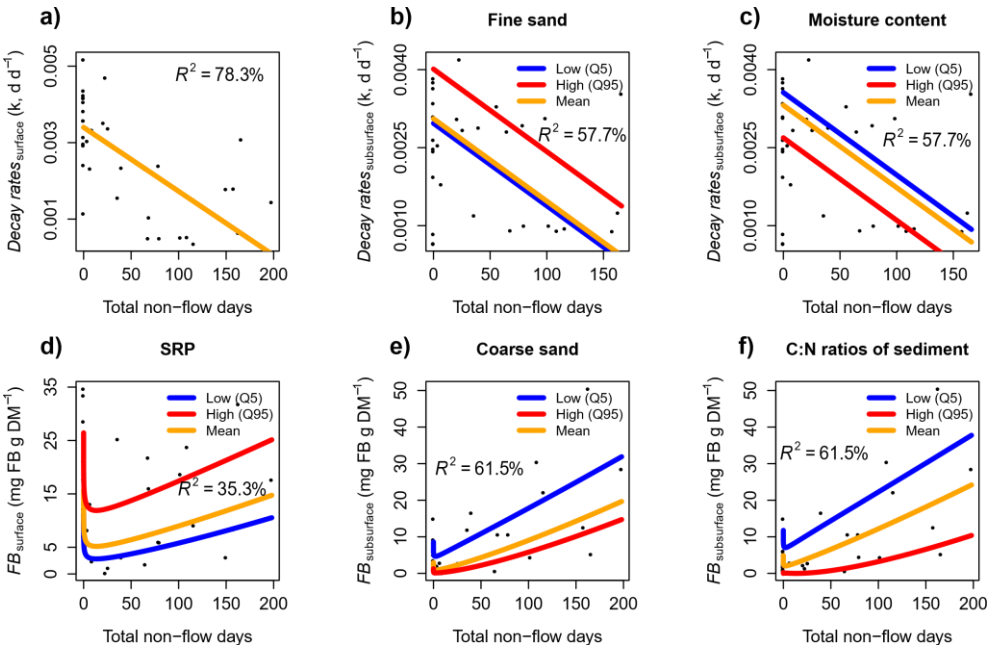


Figure 15. Responses of OM decay rates (a, b, c) and fungal biomass (d, e, f) to hydrological and environmental predictors using LMM and LMs. Fitted lines are shown for OM decay rates and fungal biomass in response to total non-flow days. Different colours represent different levels (Q5, Q50, Q95) for the variable not shown in the abscise axis (i.e., b fine sand; c moisture content; d SRP; e coarse sand; and f C:N ratios of the sediment): red represents large values (Q95), orange represents the median value (Q50) and blue represents low values (Q5), within the data set.

Table 5. Results of LMM for decay rates on the surface zone and LMs for decay rates on the subsurface zone and fungal biomass in both the surface and subsurface zones. Standardised effect sizes (SES), and their standard errors (SEs) and p-values are shown. Significant variables ($p \leq 0.05$) are highlighted in bold.

	Predictors	SES	SE	p-value	Explained variance	R^2m	R^2c
Surface OM decay rate, K (dd^{-1})	Intercept	0.00	0.00	<0.00		54.2	78.4
	TNF	-0.00	0.00	**<0.00**	51.3		
	SRP	-0.00	0.00	0.28	2.2		
	Conductivity	0.00	0.00	0.91	0.0		
Surface Fungal Biomass (mg FB g DM^{-1})	Intercept	2.35	0.44	**<0.00**		35.2	
	TNF	-0.14	0.23	0.54	3.8		
	TNF^2	1.03	0.42	**0.02**	17.0		
	SRP	0.56	0.21	**0.01**	15.3		
	Conductivity	0.23	0.22	0.31	2.2		
Subsurface OM decay rate, K (dd^{-1})	Intercept	0.00	0.00	<0.00		59.6	
	TNF	-0.00	0.00	**<0.00**	46.1		
	Fine sand	0.00	0.00	**0.02**	4.6		
	Moisture content	-0.00	0.00	**0.00**	11.5		
	C:N sediment	0.00	0.00	0.55	0.4		
Subsurface Fungal Biomass (mg FB gDM^{-1})	Intercept	1.47	0.36	<0.00		61.5	
	TNF	0.97	0.23	**<0.00**	27.3		
	TNF^2	1.19	0.34	**0.00**	19.2		
	Coarse sand	-0.65	0.22	**0.01**	1.6		
	Moisture content	-0.06	0.24	0.78	0.0		
	C:N sediment	-0.78	0.21	**0.00**	17.5		

Discussion

Overall, our findings confirm our hypothesis that subsurface processes had an important contribution to sustaining microbial decomposition during the non-flow periods of intermittent and ephemeral streams (IRES). We also showed that the magnitude of microbial decomposition and fungal biomass in the surface and subsurface zones depends on the local environmental factors of streams, such as SRP in the surface zone and sediment grain size, water content and sediment C:N ratio in the subsurface zone.

Effects of hydrology and zone on OM decomposition and fungal biomass

Previous research has shown that the duration of the non-flow period is a key factor in controlling microbial activity and OM decomposition (Bruder *et al.*, 2011; Foulquier *et al.*, 2015). However, thus far, most studies have focused on either the surface zone or the subsurface zone (Pinna & Basset, 2004; Corti & Drummond, 2011; Burrows *et al.*, 2017; Datry *et al.*, 2018b), rather than simultaneously considering both zones. Nevertheless, our results demonstrate that simultaneously studying both zones is crucial to furthering our understanding of how microbial decomposition may respond to the expected increase in the duration and severity of flow intermittency as a result of climate change. In fact, although OM processing on both zones decreases with increased intermittency, the subsurface zone could maintain OM decomposition when the period without flow lengthens **(Figures 13a and 14a)**; for instance, streams with more than 75 days of non-flow could

maintain approximately the same decomposition rates as the surface zones in streams with less than 75 days of non-flow.

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

On the other hand, OM decay rates are higher in the subsurface zone than in the surface zone as the number of non-flow days increases, this could be due to fungal biomass in the subsurface zone increasing when the intermittency increases **(Figure 13b)**; therefore, the subsurface zone could potentially maintain OM decomposition during non-flow periods. This

confirms our hypothesis that the subsurface zone is active over an intermittency gradient and reinforces the results of Burrows et al., (Burrows *et al.*, 2017) who found a similar trend using a qualitative approach (permanent *vs.* intermittent streams) in Australian streams.

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

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

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

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

In the subsurface zone, our results suggest that microbial decomposition depends on the combined influence of hydrology and sediment characteristics such as C:N content, grain size and porosity (Artigas *et al.*, 2008; Medeiros *et al.*, 2009; Cornut *et al.*, 2010; Mora-Gómez *et al.*, 2018). Fine sand can retain water for longer and thus could favour the growth of microbial decomposers (mainly bacteria), which translates into higher decay rates (Ghate & Sridhar, 2015), as we observed in this study.

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

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

Conclusions

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

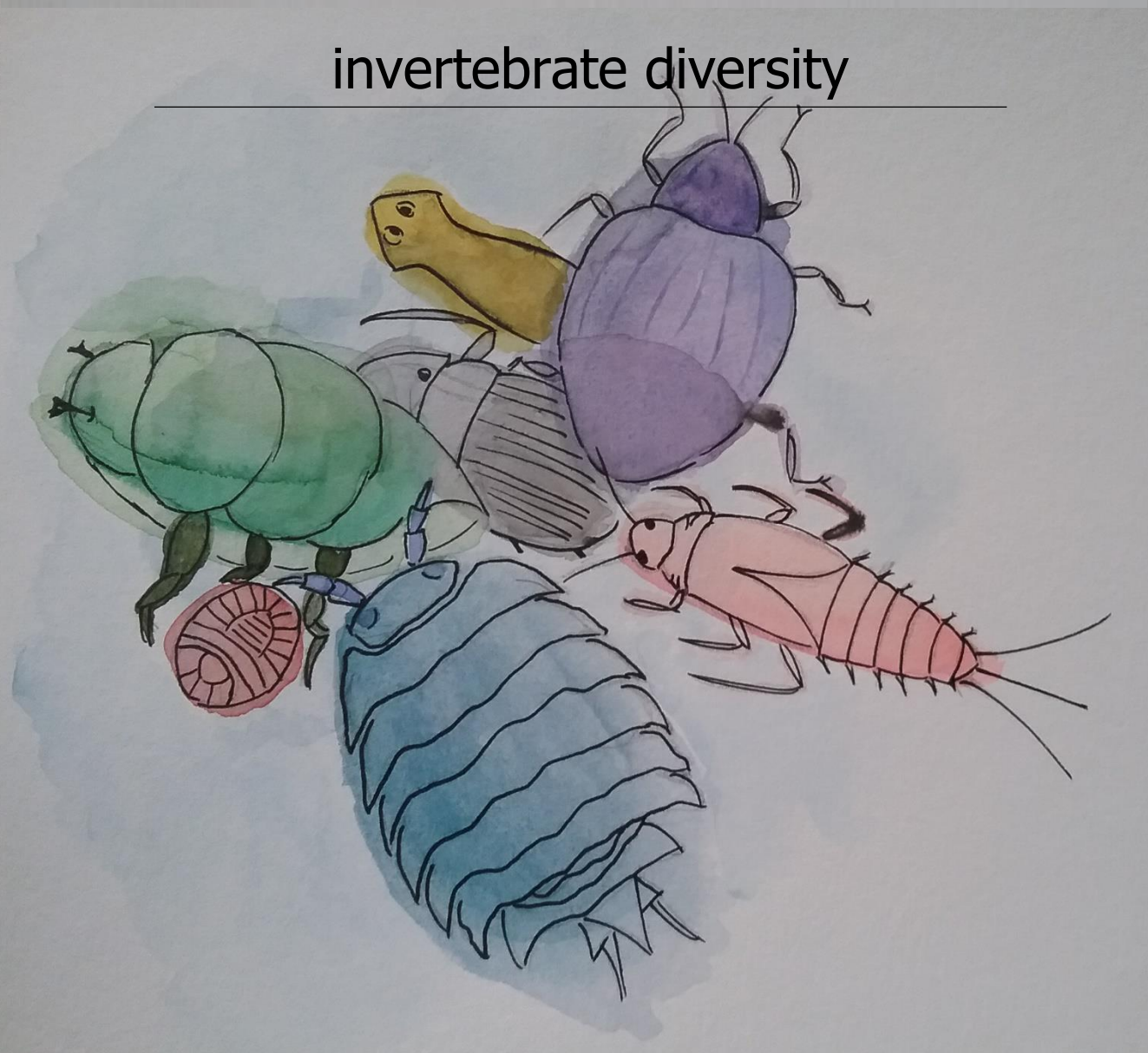
Altogether, these findings indicate that dry streambeds must be considered to ensure the fluvial ecosystem functions carried out by sediment microbiota.

Acknowledgements

We gratefully acknowledge the field survey assistance of Giulia Gionchetta and Miriam Colls.

CHAPTER 2

Annual and recent flow-intermittence disturbances explain the high variability of stream invertebrate diversity



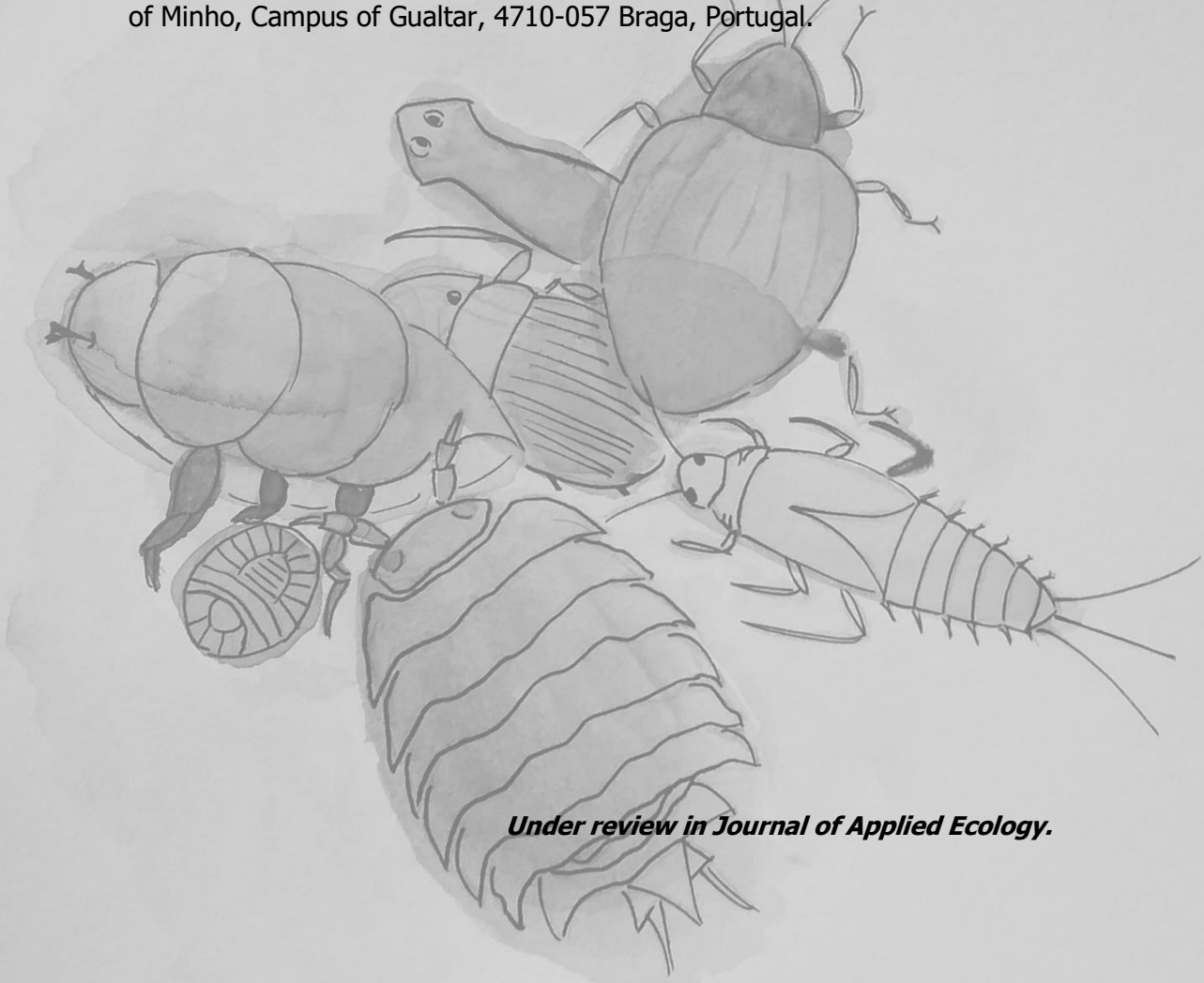
Annual and recent flow-intermittence disturbances explain the high variability of stream invertebrate diversity.

Rebeca Arias-Real[1], Cayetano Gutiérrez-Cánovas[2,3], Margarita Menéndez[1], Verónica Granados[1] and Isabel Muñoz[1].

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Barcelona, Spain.

[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

[3] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.



Under review in Journal of Applied Ecology.

Abstract

In highly dynamic ecosystems, such as intermittent and ephemeral streams, annual and recent temporal scales of disturbance set two sequential environmental filters that influence the type of organisms that can thrive. However, the exploration of how temporal disturbance scaling influences biological communities remains elusive, limiting our capacity to predict the trajectories of these ecosystems in response to global change. Using invertebrate communities from 33 streams and a comprehensive in situ hydrological characterization, we assessed the effects of two temporal scales of flow-intermittence disturbance (annual regime and recent status) and non-hydrological factors (geomorphology, water chemistry, land-use) on multiple stream biodiversity aspects (taxonomic indices and trait-based metrics), which relate to community assembly and ecosystem functioning. The annual regime metrics cover the annual duration and frequency of non-flow periods, while recent status metrics include the durations of the last non-flow period and last rewetting event. Our results showed that long periods or high frequencies of non-flow days and short rewetting periods reduced the diversity and abundance of invertebrate communities and trophic groups. Among trophic groups, predators, grazers, shredders and filterers showed the most pronounced responses to intermittency. Overall, hydrological filters outweighed non-hydrological factors in explaining invertebrate community variation. However, a combination of annual and recent scales of flow intermittence was needed to explain the highly dynamic stream invertebrate communities and, potentially, their ecosystem functioning. This result suggests that the focus on the widely-used annual non-flow days or non-hydrological factors when defining river typologies or designing bio-monitoring programmes would render less effective tools. Our

framework, which combined annual and recent flow disturbance measures and characterized structural and functional stream diversity, can help to improve the classification and bio-monitoring of highly dynamic waters and anticipate the ecological impacts of flow alteration and climate crises on freshwater ecosystems.

Introduction

The prediction of the impacts of global change on ecosystems and nature's contribution to people requires a better understanding of how organisms respond to natural and anthropogenic disturbances and influence ecosystem processes (Baert *et al.*, 2016; Palmer & Ruhi, 2019). Long-term exposure to chronic, predictable and even episodic disturbances, such as fire, drought or flood, has promoted the evolution and development of biological and functional adaptations that allow the development of life cycles under these stressful and varying conditions (Lytle & Poff, 2004; Buchwalter *et al.*, 2008; Bowman *et al.*, 2009). These fluctuating habitats can show great spatial and temporal variability in community composition, hindering our ability to detect anthropogenic impacts and apply bio-monitoring routines (Ruhí *et al.*, 2017; Rolls *et al.*, 2018; Soria *et al.*, 2020). Regarding temporal scales, annual and recent disturbance dynamics set two sequential environmental filters, i.e., the disturbance regime and the disturbance status, which influence the type of organisms that can thrive in highly dynamic environments (Tonkin *et al.*, 2017; Rolls *et al.*, 2018). First, the disturbance regime determines the number of days in which abiotic conditions allow the colonization and development of organisms in a given habitat (e.g., number of fire, drought or flood events within a period of time). This chronic filter

selects organisms that cannot complete their life cycle under such conditions during any season, distinguishing between tolerant and intolerant organisms (Bruno *et al.*, 2016a). Second, the disturbance status varies seasonally and provides information about recent disturbance events that determine the moment when habitats can be colonized and the window for growth and reproduction (e.g. time since the last fire, drought or flood). This seasonal filter sets when well-adapted, tolerant organisms can colonize a given habitat and the time they had to grow and reproduce (McMullen *et al.*, 2017). However, despite their potential implications, the role of temporal scaling in structuring the biological communities of highly dynamic ecosystems remains elusive, limiting our capacity to predict the trajectories of these ecosystems in response to global change.

A well-known, global example of a fluctuating habitat is streams that naturally and eventually experience periods of complete flow disruption -in time or space- (Datry *et al.*, 2017b). Such intermittent and ephemeral streams (IRES) represent roughly half of the global river network, although their spatial extent is expected to increase as a result of climate change and water abstraction (Scheider *et al.*, 2017). One of the main limitations to assessing the ecological status of such streams, as required by national laws, is the lack of accurate models to predict their spatial-temporal dynamics under unpolluted conditions (Stubbington *et al.*, 2018), due to an incomplete characterization of the intermittence regime and status. Thus, although IRES are likely to be much more responsive to variations in continuous hydrological descriptors of the intermittence regime (disturbance regime) or status (disturbance status) (Rolls *et al.*, 2018; Soria *et al.*, 2020), other less desirable descriptors are commonly used in bio-monitoring and conservation programmes. So far, the most common approach to characterize flow intermittence is based on categorical classes, e.g. permanent, intermittent,

ephemeral stream, or connected vs. disconnected status (Gallart *et al.*, 2012, 2017) or through mean annual values of non-flowing days obtained from hydrological models (e.g. Belmar et al., 2019). Moreover, bio-monitoring programmes, such as the Water Framework Directive (WFD), have defined river and stream typologies using coarse climatic, geomorphological, land-use and water chemistry variables (non-hydrological filters) and, only in some cases categorical descriptors of the hydrological regime (Stubbington *et al.*, 2018). These approaches could have a limited capacity to predict community responses by not considering the continuity of intermittence or by ignoring inter-annual variability or the recent intermittence events and their duration (Poff, 1997; Patrick & Yuan, 2017). To overcome these limitations, a novel and low-cost way to characterize flow intermittence through continuous temperature data-loggers has opened a promising avenue to better characterize hydrological filters and their capacity to capture community responses (Patrick & Yuan, 2017; Muñoz *et al.*, 2018), yet their predictive potential is still unknown.

Alongside the incomplete characterization of intermittence, stream communities have been only partially described. Studies published so far have focused on taxonomic aspects that consider species as functionally equivalent (e.g., taxon richness or abundance), which do not allow inference of their mechanistic relationship with environmental filters and ecosystem functioning (Suding *et al.*, 2008). The use of the response-effect trait framework in invertebrates offers a good opportunity to better understand these links (Bruno *et al.*, 2016a; Soria *et al.*, 2020) by distinguishing morphological, behavioural or life-history traits to cope with hydrological filtering (response traits) and other aspects (e.g. functional feeding strategies) that support ecosystem functioning (effect traits). Considering the key role of biodiversity in ecosystem functioning (Tonkin *et al.*, 2017;

Rolls *et al.*, 2018; Palmer & Ruhi, 2019), hydrological filters are expected to influence the multiple functions provided by streams through changes in the diversity of response and effect traits and composition of aquatic organisms.

Here, using invertebrate communities from 33 streams along a wide flow-intermittence gradient, we assessed the effects of the annual regime and the recent status of intermittence disturbance on multiple biodiversity components, including both taxonomic and functional trait-based metrics, i.e., based on either response or effect traits. We also assessed the effects of non-hydrological filters (climate, geomorphology, land-use and water chemistry), which are typically used to classify and predict community composition in bio-monitoring programmes such as the WFD (Stubbington *et al.*, 2018). To do so, (1) we deeply characterized the stream hydrology for one year before the sampling date through metrics describing the intermittence regime (total non-flow days and number of non-flow events) and intermittence status (duration of the last non-flow period and time since rewetting). Afterwards, (2) we assessed how these intermittence filters (i.e., regime and status), together with non-hydrological filters, influence whole-community taxonomic and trait-based metrics, i.e., richness, Shannon diversity, total abundance, functional diversity, and functional redundancy. Finally, (3) we explored how trophic groups responded to hydrological and non-hydrological filters by investigating changes in their abundances and response trait diversity. Given their expected influence on stream biodiversity (Tonkin *et al.*, 2017; Rolls *et al.*, 2018; Palmer & Ruhi, 2019), we hypothesize that hydrological filters will account for more taxonomical and functional variation than non-hydrological filters. In addition, with these results, we expect to provide managers and researchers a tool for determining the aspects of flow intermittence that are most important for structuring aquatic

life in IRES and open a new window for better classification and bio-monitoring of highly dynamic water courses.

Materials and methods

Study site

This study was conducted in 33 streams that belong to nine different basins across Catalonia (NE Spain) **(S2-Figure 2)**. The primary land-uses at the riparian scale were forest, scrubland, grasslands and extensive agriculture (mainly olive groves and vineyards) (Corine Land Cover 2006 data in a buffer area of 1 km around each sampling site) **(S2-Table 7)**. The studied streams have orders ranging from two to four over an altitudinal range of 81 to 920 m.a.s.l. The climate is typically Mediterranean with dry and warm summers, and precipitation mainly occurs during spring and autumn.

Characterization of hydrological filters

We characterized the metrics describing two sequential hydrological filters that operate over a hydrological year as a result of flow intermittence: (i) Intermittence regime metrics that provide information about hydrological disturbances that determine whether organisms can colonize and complete their life cycle within a hydrological year (primary hydrological filter). This type of metric includes the total number of non-flow days (TNF) and the number of non-flow periods (NFP) during one hydrological year. (ii) Intermittence status metrics that account for recent hydrological

disturbances and recovery events, which influence the colonization, development and reproduction of organisms that can thrive within a given intermittence regime (secondary hydrological filter) **(Figure 16a)**. Intermittence status metrics include the duration of the last non-flow period (NF) and the number of flowing days (rewetting, hereafter termed RE) since the last non-flow period **(Figure 16b).**

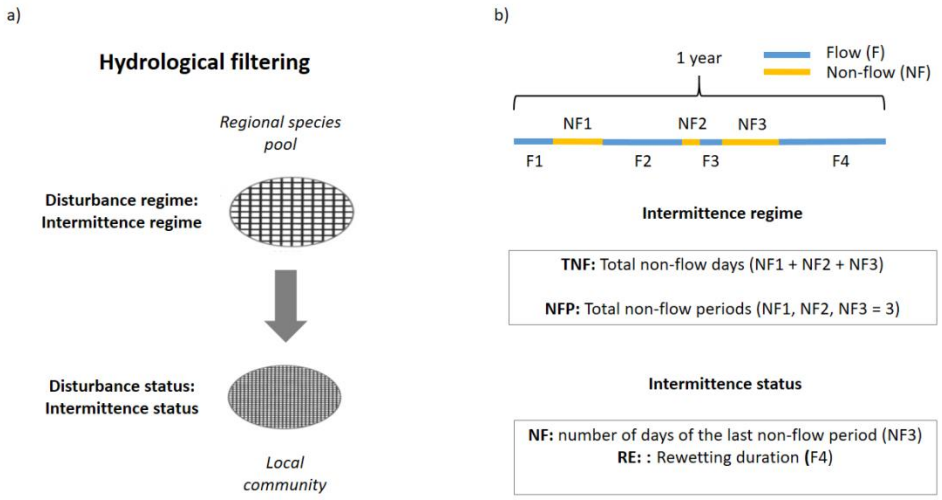


Figure 16. (a) Conceptual framework of how hydrological filtering acts on invertebrate biodiversity and, (b) example of the calculation of the different metrics for each hydrological filter used in this study. Metric acronyms: TNF, total non-flow days; TF, total flow days; NFP, non-flow periods; NF, days of the last non-flow period; RE, rewetting duration.

To quantify these metrics, we placed leveloggers (Solinst Levelogger Edge; full-scale reading precision of 0.05%) on the streambed to record the water level and temperature as proxies of the presence or absence of flow (Constantz *et al.*, 2001). The leveloggers measurements were recorded at

hourly intervals for one year (study period from January 2016 until February 2017). The recorded data were corrected for barometric atmospheric pressure variations using data from barologgers (Solinst Barologger; full-scale reading precision of 0.05%) installed at each site in the riparian area.

To calculate the flow-intermittence metrics, we also used the daily variation in the streambed temperature, i.e., the difference between the maximum and minimum temperature per day and the daily highest rate of change per hour. We calculated the fifth-order moving average to smooth the daily differences in temperature. We standardized each value with a fixed value per month. We calculated the fixed value using data from field observations, data from the meteorological stations (Servei Meteorològic de Catalunya) at each site (or nearby from each site) and the water level data from the leveloggers. Furthermore, we corrected the occasional similarity between streambed temperature and air temperature during autumn and spring with precipitation data from the meteorological stations (Arias-Real *et al.*, 2020; Gionchetta *et al.*, 2020).

Characterization of the non-hydrological filter

To represent non-hydrological filtering, for each stream location, we gathered climatic (annual precipitation, water temperature), geomorphologic (altitude, basin area, stream order), land-use (percentage of natural, agricultural and urban land-uses in the stream basin) and water chemistry (water electrical conductivity, pH, dissolved oxygen and nutrient concentrations) data (see **S2-Environmental characterization** for methodological details).

To create a variable that synthesizes all of these non-hydrological environmental descriptors, we performed principal component analysis (PCA)

based on the climatic, geomorphologic, land-use and water chemistry variables. We retained the first PCA axis (27.3% of the initial variance), which was positively related to rainfall, natural land-use and high water oxygen concentration and negatively related to high water conductivity and agricultural land-use **(S2-Table 8 and Figure 3)**.

Invertebrate data collection

At each stream location, we collected invertebrate samples just after the rainy season (February 2017) to ensure that all streams were in flowing phase, i.e., all mesohabitats were available and connected ("eurheic" status, *sensu* Gallart et al., 2012). To characterize both invertebrate abundance and diversity, we collected samples using kick-net and quantitative Surber samples, respectively. Kick-net samples (500-µm mesh) were collected through a multi-habitat standardized protocol, with sampling effort proportional to each habitat occurrence (Jáimez-Cuéllar *et al.*, 2002) and with a duration of five minutes. Kick-sample contents from each location were pooled into a unique sample for each site. We also collected three Surber samples (area: 400 cm^2, mesh size: 250 µm) in riffle areas. All samples were preserved in formalin (4%). Specimens were identified and counted in the laboratory at the genus level for most taxa or at the family level in a few cases (e.g., Diptera). Hirudinea, Oligochaeta, Ostracoda, and Hydracarina taxa were excluded from analyses because trait information was unavailable or incomplete (Tachet *et al.*, 2002).

Response and effect traits

To characterize the invertebrate traits for 67 taxa, we compiled a database including four response traits (lifespan, number of generations per year, reproduction and respiration) (Tachet *et al.*, 2002; Bonada & Dolédec, 2011) and one effect trait (trophic preferences) (http://www.freshwaterecology.info; Moog, 2002; Schmidt-kloiber & Hering, 2015). Both response and effect traits were fuzzy coded, which means that for each invertebrate taxon, a degree of affinity (i.e., ranging from 0 to 10) was assigned to each trait category according to the frequency of occurrence within the genus. Prior to analysis, fuzzy coded data were converted into percentages of affinity for each trait.

The four selected response traits were potentially related to resilience and resistance to flow intermittence: (i) short lifespan; (ii) multivoltinism; (iii) ovoviparity and terrestrial reproduction; and (iv) aerial respiration (for more details see **S2-Table 9**). Effect traits are represented by trophic preferences that support key stream functions, such as energy and organic matter consumption and transfer to higher trophic levels, nutrient cycles and secondary production (Arias-Real *et al.*, 2018; Palmer & Ruhi, 2019). Based on trophic preferences, we defined six trophic groups that included taxa showing at least 50% trophic specialization for a given feeding mode: filterers (FIL), gathering-collectors (GAT), grazers (GRA), predators (PRE), shredders (SHR) and omnivores (OMNI) when they did not show a sufficient degree of specialization for a given mode (less than 50%).

Biodiversity metrics

We used kick-net invertebrate samples to characterize presence-absence, richness-type metrics (hereafter, kick) and Surber quantitative invertebrate samples when abundance was utilized (hereafter, Surber). We calculated three types of taxonomic metrics describing: invertebrate richness (Ric, kick), total abundance (Abun, Surber) and the effective number of taxon diversity based on the exponential Shannon diversity index (Sha, Surber) (Jost, 2006). The three measures were combined as taxonomic diversity metrics (TD).

As community-level trait-based metrics, we calculated descriptors quantifying the range and diversity of response (R) and effect traits (trophic preferences, T) using functional richness (response richness: R-FRic and trophic richness: T-FRic, kick) (Villéger *et al.*, 2008) and the abundance-weighted functional dispersion index (response diversity: R-FDis and trophic diversity: T-FDis, Surber) (Laliberte & Legendre, 2010). We also estimated the community-level functional redundancy as the mean number of individuals per trophic group, i.e., total abundance divided by the number of trophic groups; FR, Surber (Bruno *et al.*, 2016a). We estimated the functional redundancy of each trophic group as the number of individuals belonging to that group that occurred in a given community (e.g., FR gathering-collectors; Surber) and the within trophic-group response diversity, as the abundance-weighted functional dispersion based on the response traits of these individuals **(Figure 17)** (Elmqvist *et al.*, 2003). All these functional measures were combined as functional diversity metrics (FD). Methodological details about trait-based metric calculations are available in **S2-Functional space.**

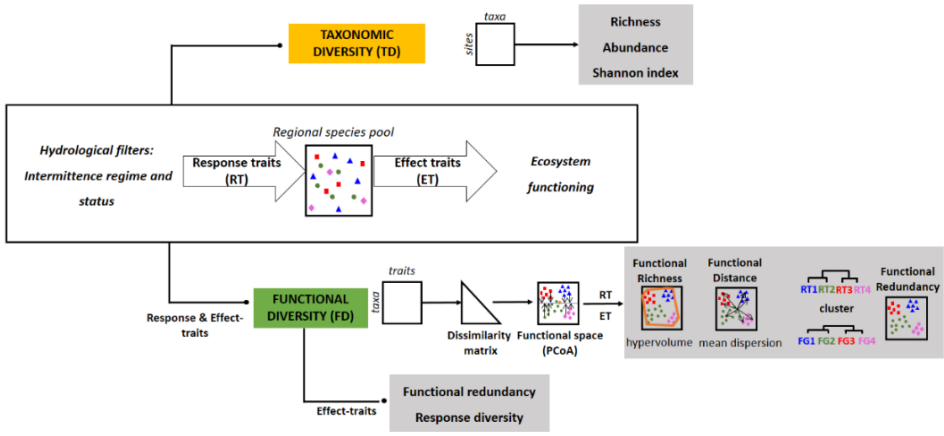


Figure 17. Scheme illustrating the taxonomic and trait-based biodiversity metrics used in this study.

Data analysis

To reduce distribution skewness, before analyses, NF, NFP, richness, and abundance were squared-root-transformed, functional redundancy measures of predation, grazing, shredding and filter feeding were log-transformed, and RE was fourth-root-transformed. All hydrological and non-hydrological filter metrics were z-standardized (mean=0, SD=1) to allow for the comparison of model coefficients. To assess predictor collinearity, we used the Variance Inflation Factor (VIF; vifstep, usdm R package) (Zuur, Ieno, & Elphick, 2010) and pairwise correlations between all potential model predictors through Pearson coefficients (cut-off of $r \leq |0.70|$) (Zuur *et al.*, 2010).

To analyse the effects of the different hydrological and non-hydrological filters on taxonomic and trait-based metrics, we used linear regression

models and a multi-model inference approach (Burnham & Anderson, 2002). First, we built 12 linear regression models (LMs) for each taxonomic and functional metric, including different combinations of hydrological and non-hydrological filters as predictors (**S2-Table 12**). All these models contained the non-hydrological filter (PCA axis 1, based on climatic, geomorphologic, land-use and water chemistry data) to quantify the effect of non-hydrological environmental variation among sites either alone (model 0) or in combination with hydrological filters (models 1 to 11). These alternative models allowed us to explore the predictive capacity of varying aggregations of hydrological and non-hydrological filters and to avoid high model collinearity (TNF was highly correlated with NF, r=0.72, **S2- Table 13**).

Second, based on the second-order Akaike Information Criterion (AICc) for small sample sizes, we ranked the 12 alternative models according to their AICc values and retained those with an AICc difference ≤ 2 with respect to the highest-ranking model. We also derived the model explained variance (R^2) and Akaike weights to determine the explanatory capacity and the relative likelihood of each model, respectively. Furthermore, we applied variance partitioning analysis (Hoffman & Schadt, 2016) to evaluate the separate contributions of hydrological and non-hydrological filters in explaining community responses. In all cases, model residuals were visually assessed to verify linear model assumptions (Zuur *et al.*, 2010).

We performed null model analysis to evaluate the degree to which trait-based metrics were independent from the underlying taxonomic variation and reflect true abiotic filtering (Veech, 2012). We then randomized the trait matrices and trophic groups (999 runs), re-calculated the trait-based community metrics (response and trophic functional richness and distance and functional redundancy) and re-ran the highest ranked models to

compare randomization-resulting and empirically observed model coefficients. We evaluated the chance for the observed value to be within the distribution range of the randomized null distribution (direct test) and within the 95% confidence interval of the randomized null distribution mean (indirect test, one sample t-test) (Veech, 2012).

All statistical analyses were performed using R statistical software version 3.4.1 (R Development Core Team, 2011).

Results

Effect of the hydrological and non-hydrological filters on whole-community metrics

TNF and NFP had a negative effect on community metrics, with the exception of Shannon index and trophic richness (T-FRic), as this intermittence regime filter was not included within the top models (**Table 6 and Figure 18**). RE had a positive influence on community metrics, except for abundance, response dispersion (R-FDis) and functional redundancy (FR), and this predictor did not appear within the top ranking models. NF was rarely included in the top models and showed both positive and negative effects. Overall, the models that were ranked first frequently contained only one hydrological predictor (e.g., TNF or RE), although some top ranking models also included models containing two hydrological predictors.

Table 6. Ranking of linear models relating hydrological (intermittence regime and status) and non-hydrological filters with species richness (Ric), Shannon diversity index (Sha), abundance (abun), response and trophic richness (R-FRic and T-FRic respectively), response and trophic diversity (R-FDis and T-FDis respectively) and functional redundancy (FR). Models are ranked based on AIC values and only those with ΔAICc ≤ 2 are shown. Columns represent the predictor effect size of the non-hydrological environmental descriptor (ENV) and the metrics characterizing hydrological filters (see **Figure 16** for acronyms). Goodness of fit (R^2) and weight are also shown. TD: taxonomic diversity metrics, FD: functional diversity metrics.

			Non-hydrological	Intermittence regime		Intermittence status					
Community metric		Model	ENV	TNF	NFP	NF	RE	R^2	AICc	ΔAICc	Weight
TD	Ric	1	0.41	-0.37				42.5	74.68	0.00	0.49
	Ric	8	0.39	-0.27			0.19	44.9	75.49	0.81	0.33
	Ric	5	0.40				0.33	39.3	76.58	1.90	0.19
	Sha	0	1.08					20.8	147.63	0.00	0.60
	Sha	5	0.99				0.48	24.3	148.42	0.78	0.40
	Abun	1	-1.00	-12.41				25.9	300.28	0.00	1.00
FD	R-FRic	5	0.05				0.07	31.6	-34.12	0.00	0.21
	R-FRic	10	0.04		-0.04		0.05	35.9	-33.93	0.20	0.19

R-FRic	2	0.05		-0.07			30.6	-33.59	0.53	0.16
R-FRic	8	0.05	-0.04			0.05	34.5	-33.21	0.91	0.14
R-FRic	9	0.05		-0.06	-0.03		33.5	-32.65	1.48	0.10
R-FRic	1	0.05	-0.06				28.7	-32.64	1.48	0.10
R-FRic	3	0.05	-0.03	-0.04			32.9	-32.31	1.81	0.09
T-FRic	6	0.09			0.06	0.08	40.5	-27.29	0.00	0.55
T-FRic	5	0.09				0.05	35.7	-26.91	0.38	0.45
T-FRic	0	0.09					29.1	-25.86	1.41	0.22
R-FDis	2	0.01		-0.01			34.9	-145.46	0.00	1.00
T-FDis	1	0.03	-0.02				40.6	-107.67	0.00	0.23
T-FDis	2	0.03		-0.02			39.8	-107.2	0.47	0.18
T-FDis	5	0.03				0.02	39.1	-106.76	0.91	0.14
T-FDis	8	0.03	-0.01			0.01	42.4	-106.41	1.26	0.12
T-FDis	3	0.03	-0.01	-0.01			42.2	-106.28	1.38	0.11
T-FDis	10	0.03		-0.01		0.01	41.9	-106.12	1.55	0.11
T-FDis	9	0.03		-0.02	-0.01		41.8	-106.09	1.58	0.10
FR	1	-0.80	-4.95				24.9	241.32	0.00	1.00

Hydrological filters were generally more important than the non-hydrological environmental filter in explaining community responses, except for Shannon diversity, trophic richness (T-FRic) and response diversity (R-FDis) (**Figure 18 and S2-Table 14**). TNF was particularly important in explaining community abundance and functional redundancy (FR), while NFP was more relevant in accounting for the variations in response richness and diversity (R-FRic, R-FDis) and trophic diversity (T-FDis). RE was the most explanatory hydrological predictor of community richness, Shannon diversity and trophic richness (T-FRic), whereas NF was of minor importance. Null model analyses revealed a significant departure of the observed model coefficients from their null distribution means for all community metrics, but the observed coefficients were within the distribution range of the null distributions (see **S2-Table 15**).

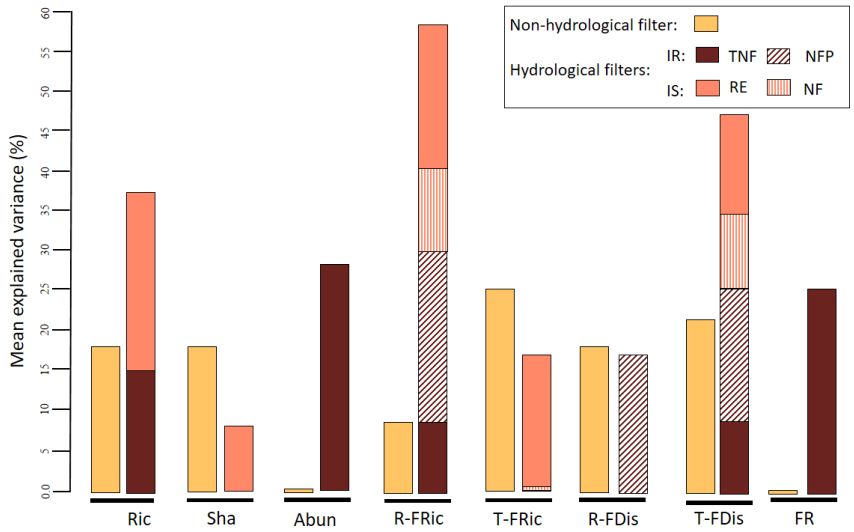


Figure 18. Bar plot with the mean explained variance (percentage, %) of all the models for each hydrological filter (intermittence regime and status) and non-hydrological filter on the different diversity metrics. See **Table 16** and **Figure 6** for abbreviations.

Effect of the hydrological and non-hydrological filters on trophic group functional redundancy and response diversity

A sharp reduction in the trophic space, defined as the hypervolume filled by a given community, was observed with intermittence. This finding highlights that flow intermittency tends to extirpate some trophic strategies (shredder, filter-feeder and predator taxa, in this example: **Figure 19, S2-Funtional space**).

The functional redundancy and response diversity of trophic groups generally declined in response to increasing TNF or NFP, while RE tended to have a positive effect (**Table 7, S2-Table 16**). NF was rarely included within the top models. Hydrological filters were more important than the non-hydrological environmental filter in explaining functional redundancy and the response diversity patterns, except for omnivores (functional redundancy and response diversity). TNF was the most important predictor of trophic group functional redundancies, except for shredders, where NFP was of great importance. For the trophic group response diversity, TNF was again the most important predictor for gathering-collectors and filterers, NFP was most important for shredders and RE was most important for predators **(S2-Figure 4)**.

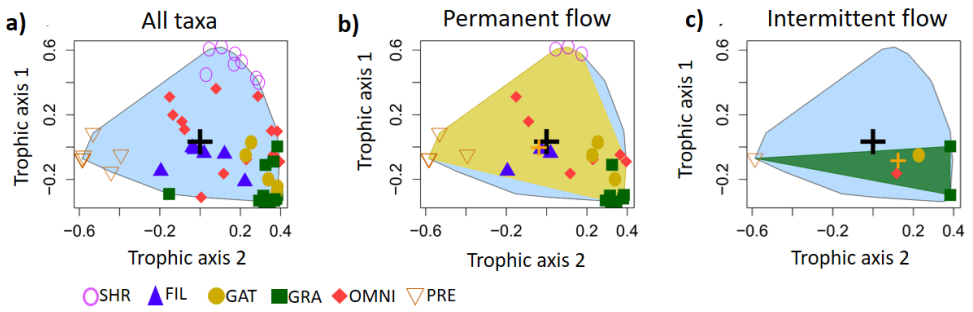


Figure 19. Illustration of how intermittence impacts trophic diversity. The 67 taxa collected in this study were distributed in different regions of the two first axes of the trophic space, according to their trophic preferences (a). Rivers with permanent flow (b) hold a greater richness of trophic groups than any given IRES (c). Coloured symbols (circles, triangles and squares) represent the different trophic groups. The black cross represents the centroid for all studied taxa (mean position along each axis), while the yellow cross shows the community centroids for rivers with permanent (b) and intermittent flow (c). Gat: gathering-collectors, Pre: predators, Gra: grazers, Shr: shredders, Omni: omnivores, Fil: filterers.

Table 7. Ranking of linear models relating hydrological (intermittence regime and status) and non-hydrological filters with the functional redundancy (FR) and response diversity (R-FDis) of the trophic groups, following an AIC-based model selection procedure (ΔAICc ≤ 2). Models are ranked based on AIC values and only those with ΔAICc ≤ 2 are shown. Columns represent the predictor effect size of the non-hydrological environmental descriptor (ENV) and the metrics characterizing hydrological filters (see Table 1 and Fig 4 for acronyms). Goodness of fit (R^2) and weight are also shown. Gat: gathering-collectors, Pre: predators, Gra: grazers, Shr: shredders, Omni: omnivores, Fil: filterers.

			Non-hydrological	Intermittence regime		Intermittence status					
		Model	ENV	TNF	NFP	NF	RE	R^2	AICc	ΔAICc	Weight
FR	Gat	0	125.48					3.32	528.58	0.00	0.45
	Gat	1	101.89	-165.02				8.71	529.15	0.55	0.35
	Gat	6	119.22	-275.32	-179.90			12.5	530.32	1.72	0.19
	Pre	1	0.49	-0.82				38.2	116.81	0.00	0.69
	Pre	5	0.46	-0.68			0.28	39.5	118.40	1.59	0.31
	Gra	1	0.30	-0.52				15.3	126.60	0.00	0.46
	Gra	5	0.34	-0.73			0.41	19.2	127.52	0.92	0.29
	Gra	0	0.37					5.3	127.94	1.34	0.23
	Shr	4	0.47		-0.96			23.7	146.99	0.00	0.71
	Shr	9	0.43		-0.75		0.40	25.2	148.82	1.83	0.29

	Omni	1	-521.75	-481.51				16.8	580.90	0.00	0.46
	Omni	0	-452.90					8.1	581.84	0.94	0.28
	Omni	2	-526.16				396.64	13.9	582.11	1.22	0.25
	Fil	1	0.32	-1.19				28.5	146.73	0.00	0.68
	Fil	5	0.37	-1.43			-0.45	30.3	148.27	1.54	0.32
R-FDis	Gat	1	0.05	-0.04				32.7	-55.59	0.00	0.41
	Gat	4	0.05		-0.04			30.8	-54.65	0.92	0.26
	Gat	2	0.05				0.03	29.2	-53.85	1.74	0.17
	Gat	6	0.05	-0.03	-0.01			33.7	-53.69	1.90	0.16
	Pre	5	0.06	-0.04			0.04	42.2	-41.87	0.00	0.35
	Pre	1	0.06	-0.06				38.2	-41.87	0.00	0.35
	Pre	2	0.06				0.06	37.7	-47.57	0.31	0.30
	Gra	0	0.04					17.2	-53.37	0.00	1
	Shr	4	0.03		-0.06			26.6	-42.45	0.00	0.42
	Shr	6	0.03	0.04	-0.08			30.7	-41.97	0.48	0.33
	Shr	10	0.03		-0.07	0.03		29.5	-41.38	1.06	0.25
	Omni	0	0.01					0.1	-65.42	0.00	0.69
	Omni	1	0.01	-0.01				3.6	-63.82	1.61	0.30
	Fil	1	0.00	-0.05				17.2	-49.32	0.00	1.00

Discussion

This study shows for the first time that annual and recent flow-intermittence disturbances shape different aspects of stream biodiversity, which determine how stream communities assemble and support ecosystem functioning. Our results suggest that long periods or high frequencies of non-flow days and short rewetting periods can reduce the diversity and abundance of invertebrate communities and trophic groups, with potentially negative consequences for ecosystem multifunctionality. Taken together, our findings demonstrate the importance of considering both intermittence regime and status metrics and trait-based aspects to provide a better prediction of the spatial variations in highly dynamic stream communities. For example, the utilization of a single descriptor of flow intermittence, such as the widely used total non-flow days (TNF), or the focus on non-hydrological aspects, can yield an incomplete view of the biodiversity and functional responses to intermittency. Considering the lower influence of the non-hydrological filter in our models, these results match our initial hypothesis that stream communities in IRES are more tightly linked to hydrological disturbances than geomorphological, land-use or water chemistry variables, even when this latter group of predictors is commonly used to predict and typify biological communities at reference conditions in bio-monitoring programmes, such as the WFD (Stubbington *et al.*, 2018).

Although our model results align with previous findings (Bruno *et al.*, 2016a; Aspin *et al.*, 2019; Belmar *et al.*, 2019; Soria *et al.*, 2020), our novelty was in finding that the negative effects of flow intermittence on stream biodiversity arise from two distinct hydrological filtering mechanisms related to richness- or abundance-based invertebrate aspects and the temporal scale

of the flow disturbance. In the first case, richness and diversity-type metrics, either taxonomic or functional, were explained by a combination of annual (frequency of non-flow periods and total number of non-flowing days) and recent (rewetting duration) flow-intermittence disturbances. The importance of both filters suggests that the intermittence regime and recent status control the variety of life forms that are able to colonize and thrive in IRES. For example, long or frequent non-flowing periods can limit the occurrence of certain taxa that are unable to complete their life cycles (McMullen *et al.*, 2017) or cope with chemical stress or desiccation (Stubbington & Datry, 2013; Granados *et al.*, 2020). Additionally, short rewetting periods can reduce the variety of colonizer (Booker *et al.*, 2015) or taxa recovering from dormancy or resistance stages (Stubbington *et al.*, 2016), thereby constraining taxon and trait richness. Our models showed that these are two plausible causes explaining the reduction in taxa richness and diversity and response trait richness with long non-flow periods and short rewetting lengths. However, the rewetting length could also be positively related to the availability of habitat and resources (Leigh & Datry, 2017), being more important for trophic diversity than response diversity, as shown in our models.

The second group of abundance-based measures was primarily determined by changes in total non-flowing days. This pattern reflects that the number of organisms within a community or trophic group depends on a wider temporal scale than taxon or trait richness metrics. In addition, the fact that the intermittence status was important for richness aspects, but not for abundance, suggests that rewetting events could have enabled a more diverse representation of organisms by adding only a few individuals to the community. Furthermore, in addition to intermittence regime metrics constraining the variety of taxa occurring in a river location (Poff, 1997;

Tonkin *et al.*, 2017), the number of non-flow days also influences the fitness and absolute abundance of an organism. For example, long non-flow periods tend to select a reduced set of fast-living, multivoltine organisms (Diaz *et al.*, 2008; Belmar *et al.*, 2019), which are prone to develop and maintain large stocks of individuals through various reproductive events within a year. However, in streams with long annual periods of non-flowing days, even intermittence-adapted organisms could show reduced abundances, probably, because of the narrow window for reproduction and survival (Stubbington & Datry, 2013).

The reduction in abundance and response trait richness at the community level mirrored the declining trends in trophic group abundances and response diversity, where predators, grazers, shredders and filterers showed the most pronounced responses. Trophic strategies showed differential responses to intermittency probably due to direct intolerance to harsh environmental conditions mediated by response traits (Stubbington & Datry, 2013; Aspin *et al.*, 2019) or because trophic cascading effects were due to alterations in basal resources (Kuehn, 2016; Pearson *et al.*, 2018). Additionally, when flow ceases, the range of dietary niches is progressively reduced due to alterations in the quantity and quality of food resources (Sanpera-Calbet *et al.*, 2017b). These results indicate that the reduced trophic representation and diversity in IRES could probably jeopardize their ecosystem functioning (Gagic *et al.*, 2015), as manifested in the reduced rates of organic matter decomposition (Arias-Real *et al.*, 2020) or simplified food webs (Siebers *et al.*, 2019).

Management and bio-monitoring implications

Intermittent streams are currently poorly classified and covered by bio-monitoring programmes due to the difficulty in defining reference types and accounting for their high biological variability (Stubbington *et al.*, 2018). This study contributes to solving this problem through a better characterization of hydrological filters, which can improve predictions of biological and functional responses to flow intermittence and, potentially, other anthropogenic pressures. By applying this framework, managers can estimate a range of meaningful biodiversity aspects that could serve as effective bio-indicators of intermittent stream health (Bruno *et al.*, 2016a; Soria *et al.*, 2020). Further studies should address the capacity of these trait-based metrics to anticipate functional responses in freshwater ecosystems (Palmer & Ruhi, 2019).

Our results also support the use of trait-based metrics for better management and bio-monitoring of ecosystems in a global-change context. Previous studies suggested that flow intermittence can confound bio-monitoring assessments by interacting with anthropogenic pressures (Soria *et al.*, 2020), but these studies considered a less detailed hydrological characterization. Our hydrological metrics can contribute to a better understanding of how natural and anthropogenic stressors interact to affect biodiversity and functional responses. In a world with declining freshwater availability, most rivers in the word will become intermittent due to climatic crises or water abstraction (Döll & Schmied, 2012), with important consequences for biodiversity and planetary functioning. Flow intermittence events may cause longer or more frequent non-flow periods, or reduce rewetting lengths with potentially different impacts on stream ecosystems.

Our hydrological and biodiversity metrics can also help to anticipate such ecological consequences, aiding the mitigation of negative impacts.

In conclusion, a combination of hydrological descriptors representing two temporal scales of flow-intermittence disturbance (annual regime and recent status) is needed to explain the variation in stream invertebrate community dynamics. These findings suggest that both intermittence components play different but complementary roles in structuring stream biodiversity and, consequently, in sustaining ecosystem functioning. Furthermore, according to our expectations, intermittence descriptors generally outweighed non-hydrological descriptors when explaining biodiversity aspects, supporting their use in IRES classification and bio-monitoring routines. Our framework, based on multiple intermittence measures and different characteristics of biological communities, can enable a better prediction of how stream communities respond to flow intermittency in a more arid world.

Acknowledgements

Authors thank Ariadna Fabà and Priscila N. Heredia for their help in invertebrate sample processing.

CHAPTER 3

Flow intermittence drives aquatic hyphomycete turnover and richness reduction which affects organic matter decomposition

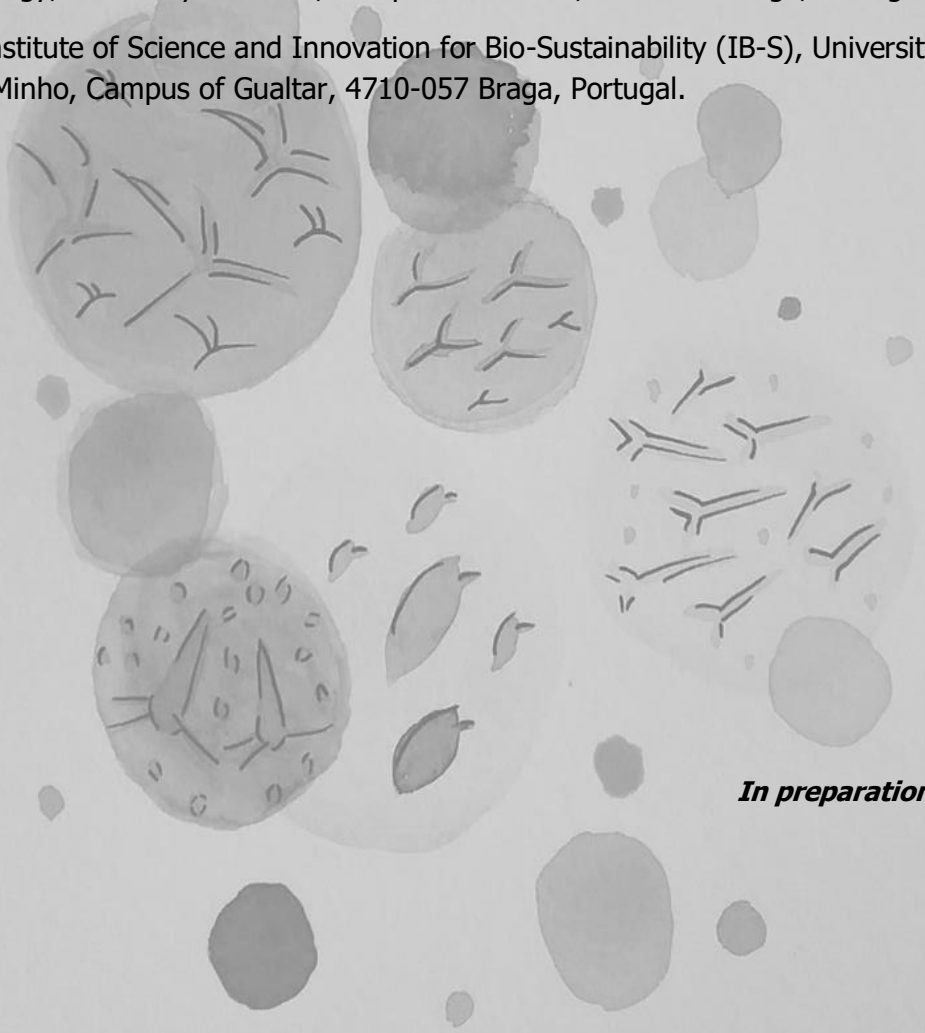
Flow intermittence drives aquatic hyphomycete turnover and richness reduction which affects organic matter decomposition.

Rebeca Arias-Real[1], Isabel Muñoz[1], Cláudia Pascoal[2,3], Cayetano Gutiérrez-Cánovas[2,3] and Margarita Menéndez[1].

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Barcelona, Spain.

[2] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

[3] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.



In preparation.

Abstract

Aquatic hyphomycetes are the main microbial decomposers of organic matter (chiefly leaf litter or woody debris) that falls into streams. They thus constitute an essential trophic link between organic matter and invertebrate consumers, and sustain fluvial food webs. We now know that aquatic hyphomycetes rely on the availability of flowing water; thus, flow cessation could lead to abrupt changes in local aquatic hyphomycete richness (alpha diversity) and changes in composition (beta-diversity). Although the prevalence of intermittent and ephemeral streams is increasing, due to climate change and water abstraction, it remains unclear how aquatic diversity is affected by and responds to flow intermittence, or how that diversity modulates the effect of flow intermittence on organic matter processing. Here, we used both observational and manipulative experiments to test the linkage between aquatic hyphomycete community alpha and beta diversity, and organic matter decomposition over a gradient of flow intermittence. Our results demonstrate that more number of non-flow days results in a reduction of aquatic hyphomycete richness and drives species turnover, indicating their resistance mechanism to flow intermittence. Furthermore, our results reveal a positive effect of aquatic hyphomycete species richness on organic matter decomposition. These findings support the idea that increased aquatic hyphomycete diversity could mediate the effects of flow intermittence on organic matter decomposition. In a context of anthropogenic global change and biodiversity reduction, our results suggest that the loss of microbial biodiversity will threaten the functioning of river ecosystems, especially in those systems that present flow intermittence.

Introduction

Microbial communities are diverse and sustain key ecosystem processes such as biogeochemical cycles involving the decomposition of organic matter or energy transfer to higher tropic levels, among other (Gessner *et al.*, 2010; Besemer, 2015). In terrestrial ecosystems, the importance of microbial diversity in driving ecosystem functioning, such as nutrients cycling, primary production, litter decomposition and climate regulation, has been demonstrated at both large spatial scales and in local manipulative experiments (Delgado-Baquerizo *et al.*, 2016; Laforest-Lapointe *et al.*, 2017; Li *et al.*, 2019). However, the role of microbial diversity in sustaining fluvial ecosystem processes is not fully yet understood (García-Palacios *et al.*, 2016; van der Plas, 2019), especially in highly dynamic water courses such as intermittent rivers and ephemeral streams (IRES). These are recognized as the most common fluvial ecosystems around the world (Datry *et al.*, 2017b) and are predicted to increase their presence due to climate change and water abstraction (Döll & Schmied, 2012; Koutroulis *et al.*, 2019).

Among microbial communities, aquatic hyphomycetes are the main biotic drivers of organic matter decomposition in their earlier stages (Cornut *et al.*, 2010): a key ecosystem process in fluvial ecosystems that constitutes an essential trophic link in detritus-based food webs (Kuehn, 2016; Arias-Real *et al.*, 2018). We already know that the decomposer activity of aquatic hyphomycetes depends on environmental factors such as temperature, dissolved nutrients and dissolved oxygen, and also on the characteristics of the substrates they colonize (e.g., type of leaf litter or wood debris) (Gulis, 2001; Bruder *et al.*, 2011). Moreover, aquatic hyphomycetes are intimately

associated with their substrate (Chauvet *et al.*, 2016), therefore they cannot evade the abrupt changes in environmental conditions when stream flow ceases, which make them particularly vulnerable to desiccation stress (Lake, 2003; Foulquier *et al.*, 2015). Thus, non-flow periods may be fatal for them, unless mycelia and propagules are protected from complete desiccation (e.g., by moving into the subsurface zone, as some aquatic invertebrates do). It is known that aquatic hyphomycetes show adaptations to flowing waters, including conidium shape (mostly tetraradiate, branched or elongated), production of mucilage that facilitates adhesion to the substrate, and rapid germination (Read *et al.*, 1992). Nevertheless, it is not clear if they present adaptations to non-flow periods, even though they have been reported in various environments other than running water, such as endophytes in roots exposed to water (Selosse *et al.*, 2008), streambed sediment (Gionchetta *et al.*, 2020), the hyporheic zone (Cornut *et al.*, 2014; Ghate & Sridhar, 2015) or leaf litter on stream banks and riparian tree canopies (Sridhar, 2009). This certainly suggest potential resistance mechanisms to desiccation (Chauvet *et al.*, 2016). Generally, the main response mechanisms of aquatic communities to flow intermittence are resistance or resilience, depending on their functional traits or phenotypes (Gutiérrez-Cánovas *et al.*, 2019). Resistance mechanisms occur when organisms are specialized for different portions of the intermittence gradient, i.e., responses are based on varying tolerance and specialization to desiccation, resulting in beta-diversity patterns explained by species turnover (Baselga, 2010; Datry *et al.*, 2014; Gutiérrez-Cánovas *et al.*, 2013; Williams, 2006). Resilience mechanisms arise when organisms cannot cope with desiccation stress and need to recolonize a given aquatic spot after rewetting, thus giving rise to nested subsets of species over the intermittence gradient (Aspin *et al.*, 2018; Chester & Robson, 2011; Datry *et al.*, 2014).

Nevertheless, and despite the increased number of studies on the factors that regulate aquatic hyphomycete diversity and activity during organic matter decomposition in streams, our knowledge of their response mechanisms to flow intermittence is limited.

Current knowledge of flow intermittence effects on the decomposition activity of aquatic hyphomycetes is based on manipulative experiments. Studies have shown that simulated flow reductions correspond to lower aquatic hyphomycete species richness (alpha diversity) as well as changes in taxonomic composition (beta diversity) and enzymatic activity (Duarte *et al.*, 2017; Mora-Gómez *et al.*, 2018). These could become translated into reduced rates of both organic matter decomposition (Gonçalves *et al.*, 2016) and the subsequent energy transfer to higher trophic levels (Arias-Real *et al.*, 2018). Nevertheless, it is not known whether these findings can be extrapolated to real systems, especially to richer communities and across larger spatial scales. Considering the global importance of IRES (Datry *et al.*, 2017b, 2018a), there is an urgent need to understand better the functional consequences of increasing flow intermittence for aquatic hyphomycetes and their role on organic matter decomposition.

Here, using both observational and manipulative experiments, we tested the linkage between aquatic hyphomycete communities and organic matter decomposition along a gradient of flow intermittence (**Figure 20**). First, based on an observational study of 15 streams, we analysed how aquatic hyphomycete richness and their taxonomic composition (through overall beta diversity together with its intrinsic turnover and nestedness components) responded over a gradient of flow intermittence. We also considered how these community changes explain variations in organic matter decomposition. Second, using a manipulative experiment, we explored joint

effects of fungal species richness and flow intermittence on leaf litter decomposition and fungal biomass. For this microcosm experimental setup, we set two levels of species richness (low and high) and three simulated levels of flow intermittence (permanent, intermittent and ephemeral). We predicted that: i) increased flow intermittence would reduce the species richness of aquatic hyphomycetes; ii) community composition changes would arise through species turnover, reflecting resistance mechanisms at the community level; and iii) higher species richness would attenuate the effect of increased flow intermittence on organic matter decomposition.

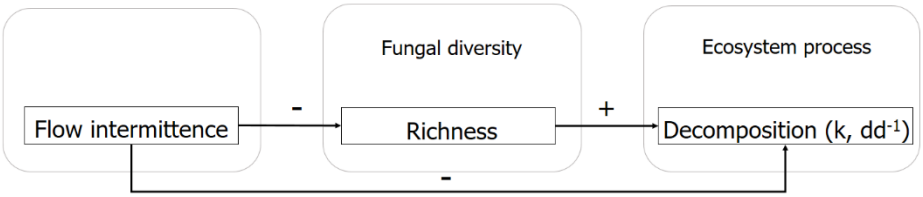


Figure 20. A priori conceptual meta-model of the relationship between intermittence, diversity and ecosystem function (B-EF). Solid lines represent hypothesized causal relationships; arrows, the direction of causality; - and +, the expected relationship: negative or positive, respectively.

Materials and methods

Field experiment

Study site

This study was conducted in 15 low-order streams that belong to eight river basins across Catalonia (NE Spain). The primary land uses at the

riparian scale were forest, scrubland, grasslands and extensive agriculture (mainly olive groves and vineyards) (CORINE Land Cover 2006 data in a buffer area of 1 km around each sampling site) **(S3-Table 17)**. Furthermore, poplar (*Populus nigra* L.), alder (*Alnus glutinosa* (L.) Gaertner) and evergreen oak (*Quercus ilex* L.O) were the dominant riparian vegetation. The climate is typically Mediterranean with dry and warm summers, and precipitation occurring mainly in spring and autumn.

Flow intermittence characterization

We calculated different metrics of flow intermittence for the selected streams based on one hydrological year. Flow intermittence was classified according to the intermittence regime metrics and intermittence status metrics. Intermittence regime metrics included information on the duration of events (total non-flow days) and their frequency (i.e., number of non-flow periods). Intermittence status metrics included information on recent events before sampling, number of days of the last non-flow period (NF) and the number of flowing days (rewetting, hereafter termed RE) after the last non-flow period up to sampling campaigns **(Figure 21)**.

To do this, we used daily variation of the streambed temperature as an indicator of water presence in lotic and lentic habitats. Daily variation was determined as the difference between the maximum and minimum temperatures on each day and the highest daily rate of change per hour. Temperature and water level were recorded with Leveloggers (Solinst Levelogger Edge, full-scale reading precision of 0.05%) that were placed on the streambed (Constantz et al., 2001). The Leveloggers recorded data at hourly intervals for one year (the study period: from February 2016 until February 2017). The recorded data were corrected for atmospheric pressure

variations using data from Barologgers (Solinst Barologger, full-scale reading precision of 0.05%) installed in the riparian zone at each sampling site. Once temperature data were retrieved, we performed a fifth-order moving average to smooth daily differences. We standardized each value with a fixed value per month, using data from field observations, data from meteorological stations (Servei Meteorològic de Catalunya; http://www.meteocat.es) near each site, and water level data from the Leveloggers. Furthermore, we corrected the occasional similarity between streambed temperature and air temperature during autumn and spring with precipitation data from meteorological stations (Arias-Real *et al.*, 2020).

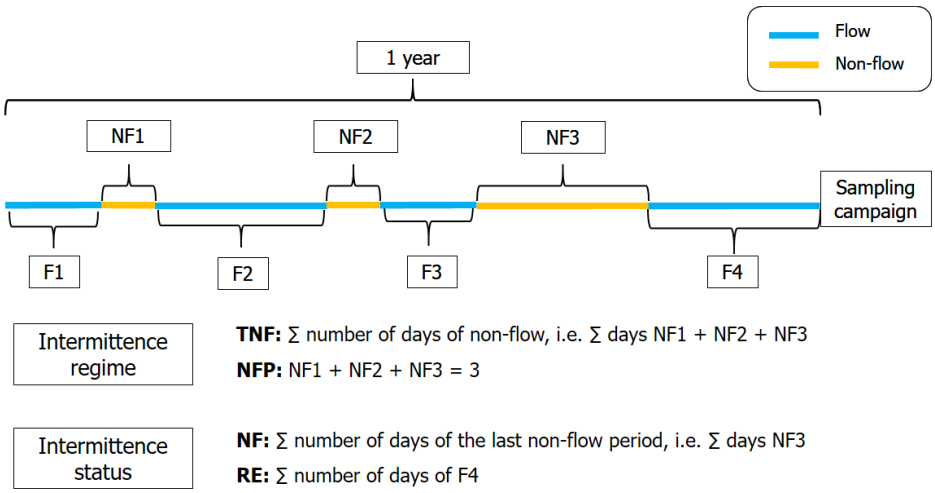


Figure 21. Example of the calculation of the intermittence regimen and the status metrics used in this study. NF is no flow, F is flow, TNF is total non-flow days, NFP is non-flow periods, NF days of the last non-flow period and RE is rewetting.

Non-hydrological characterization

For each stream location, we performed three sampling campaigns: the first during February 2016, the second during September 2016 and the third during February 2017. At each time and location, when surface water was present we measured water electrical conductivity, water temperature, pH and dissolved oxygen (±1 μs cm^{-1}, ±0.1 °C, ±0.005 pH and ±0.1 mg L^{-1}, respectively) using a portable probe (YSI Professional Plus Multiparameter Instrument, USA). Furthermore, we took water samples and characterized their nutrient concentrations: nitrite, nitrate, ammonium and soluble reactive phosphorus (SRP) (for methodological details **S3-Environmental characterization**). Climate and geomorphologic data were extracted from Servei Meteorològic de Catalunya (http://www.meteocat.es) and ACA-Agència Catalana de l'Aigua public data (http://aca.gencat.cat), respectively. Land use data were obtained from the CORINE Land Cover 2006 data, from a buffer area of 1 km around each sampling site.

To represent environmental variation, we performed principal component analysis (PCA) based on climatic, geomorphologic, land-use and water chemistry variables. We selected the first PCA axis 1 (28.7% of the initial variance) which is positively related with altitude, natural land use and higher water oxygen concentration, and negatively with higher SRP, DIN and agricultural land use (for details, see **S3-Table 18 and S3-Figure 5**).

Fungal assemblages

We sampled aquatic hyphomycete conidia just after the rainy season, in February 2017, to ensure that all the streams were in the flowing phase. We collected samples of white freshly accumulated foam from different

locations within 100 m stretches in each stream with a spoon. The foam was then transferred to sterile glass bottles (25 ml), fixed with formaldehyde (4%), and transported to the laboratory.

Once in the laboratory, 5 ml of each foam sample, with the corresponding suspensions of conidia was filtered through 5 µm pore-size membrane filters (Cellulose Nitrate Membrane Filters, Whatman). The filter was stained with one drop of Trypan Blue solution containing lactic acid. Then we scanned the surface of the filter under a light microscope (400x), and identified all conidia until the species level (74 species in total). The result were reported as the presence/absence of aquatic hyphomycete species.

Organic matter decomposition

To quantify organic matter decomposition in the field we used five sticks of *Populus canadensis* wood (15 × 2 × 0.2 cm) (Arroita *et al.*, 2012) tied to metal bars on the streambed or branches or roots, with nylon threads to ensure that they remained in the lotic habitat of each stream. The sticks were individually marked, oven-dried (70ºC, 72 h) and weighed to determine initial dry mass before being immersed in the streams. An extra set of five sticks per stream were transported but not placed in the streams, and then returned to the laboratory to correct the initial weight, taking into account losses due to manipulation. These sticks were also used to calculate the initial ash content. The sticks were immersed in each stream for one hydrological year. On retrieval, the sticks were gently brushed to remove adhered material and washed with distilled water. Afterwards, each stick was dried (70ºC, 72 h) and weighted to calculate the final dry mass. Finally, they were incinerated (500ºC, 5h) to determine the ash-free dry mass by removing the organic components. Decay rates were calculated according to the negative

exponential model (Petersen and Cummins, 1974). We expressed the decay rates in terms of accumulated heat by replacing time with the mean daily temperatures accumulated (degree-days, dd^{-1}) (Stout, 1989). To express the decay rates in degree-days, we used the mean daily temperatures from the Leveloggers.

Microcosm experiment

To ensure differences in richness and composition of aquatic hyphomycete species, we selected two streams for leaf conditioning based on our previous results of the field study (see above): one permanent stream with a high degree of species richness (site 1, hereafter termed A) and one intermittent stream with poorer species richness (site 4, hereafter termed B). Both streams are third-order streams with siliceous bedrock, meaning that despite their different flow regimes, the water they carry has similar physical and chemical characteristics (for details **S3-Analysis of water physicochemical characteristics at A and B**). We initially collected black poplar leaves (*Populus nigra L.*) freshly abscised in autumn 2016 from the riparian area of site 1 and dried them at room temperature. Sets of dried leaves (approximately 10-12) were placed in fine-mesh bags (0.5 mm mesh) and immersed for 20 days in the streams (23 mesh bags per stream) along a 100 m reach (Arsuffi & Suberkropp, 1984; Suberkropp & Arsuffi, 1984). After retrieval, the bags were transported to the laboratory, leaves were washed and 600 discs (16 mm diameter) were cut from them per stream, using a cork borer and avoiding the veins.

Microcosm setup

Before starting the microcosm assay, we characterized the initial communities of aquatic hyphomycetes on leaves previously conditioned in the two streams **(S3-Analysis of the differences between initial aquatic hyphomycete communities at A and B)**. To that end, we induced the release of conidia from the leaves by placing sets of 5 leaf discs into Erlenmeyer flasks with 60 ml of filtered (0.5 µm pore size) water from each stream at 14ºC, under a 12 h light:12 h dark photoperiod, for 48 h (10 replicates). In the flasks, the leaf discs were aerated by a continuous airflow that created turbulence and kept them in continuous motion. After 48 h, 5 ml of stream water with the corresponding suspensions of conidia was filtered, and the conidia retained in the filters were stained as described above. Each filter was scanned under the light microscope (400x) for conidium identification and the result was species presence or absence.

The initial dry mass of leaves was determined by five weighing previously lyophilized leaf discs from each stream (10 replicates). To determine the initial fungal biomass, another set of five leaf discs from each stream (5 replicates) were frozen at -80ºC until processed (see details below).

The microcosms consisted of 100 ml Erlenmeyer flasks each containing fifteen leaf discs conditioned in stream A or in stream B and 50 ml of the respective filter stream water (30 replicates per stream). The microcosms were incubated at 14ºC, under a 12 h light:12 h dark photoperiod, and continuously aerated, as described above. Microcosms from each stream (A and B) were subjected to the following three treatments **(Figure 22)**.

Treatment 1 (I) simulated an intermittent stream with 28 days with no stream water (non-flow period) and then 14 days with filter stream water

(rewetting); 10 replicates per stream. Treatment 2 (P) simulated a permanent stream with 42 days of flow, i.e., with filter stream water; 10 replicates per stream. Treatment 3 (E) simulated an ephemeral stream with 42 days without filter stream water; 10 replicate per stream.

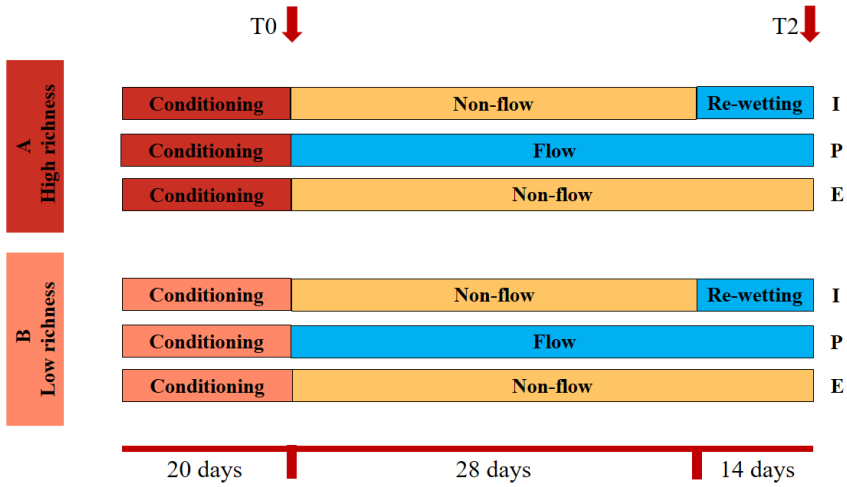


Figure 22. Experimental design. A and B refer to the leaf discs conditioned in the corresponding streams with high and low species richness, respectively. Flow intermittence treatments: I - intermittent stream, P - permanent stream, E - ephemeral stream. T0 and T2 correspond to the beginning and end of the microcosm experiment. Each microcosm contained 15 leaf discs; with ten replicates per treatment.

The non-flow period of 28 days was based on the experiment by Bruder et al., (Bruder *et al.*, 2011) to ensure all leaf discs were completely air dried. In the microcosms with stream water, this was renewed in every 3 days. Conidium suspensions from different dates were pooled and fixed with formaldehyde (4%) until identification (see below).

Leaf mass loss

We determined the percentage of leaf dry mass loss (hereafter-termed DM loss) as a proxy for leaf litter decomposition. To determine the percentage of DM loss across treatments, at T2 discs from each microcosm were lyophilized and final dry mass was determined by weighing. Leaf mass loss were calculated as the difference between initial (T0) and final (T2) dry mass.

Fungal biomass

The ergosterol concentration in the leaf discs was determined as a proxy for fungal biomass (Gessner, 2020). Sets of five frozen leaf discs were lyophilized and weighed to determine their dry mass. Lipids extracted from leaf discs and saponified in 0.14 M KOH methanol (8 g L^{-1}), at 80 °C for 30 min, in a shaking water bath. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp., Milford, MA, USA), and ergosterol was eluted using isopropanol. We used high-performance liquid chromatography (HPLC) to detect and quantify the ergosterol by measuring the absorbance at 282 nm. We used a Jasco HPLC system (USA) equipped with a Gemini-NX 5 µm C18 250 × 4.6 mm column (Phenomenex, UK). The mobile phase was 100% methanol, and the flow rate was set to 1.2 ml min^{-1}. Finally, we converted the ergosterol into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium (Gessner & Chauvet, 1993). We expressed the results in mg of fungal biomass per gram of leaf litter dry mass.

Aquatic hyphomycete richness after non-flow period in intermittent treatments

Conidium suspensions from each microcosm were identified (for details see above). We expressed the results as a conidia presence or absence matrix.

Data analysis

Field experiment

To reduce distribution skewness before analysis, NF and decay rates (k degree-days, dd^{-1}) were squared-root-transformed. Flow intermittence metrics and the non-hydrological environmental factor (ENV) were z-standardized (mean=0, SD=1) to facilitate model coefficient comparisons. To assess predictor collinearity, we used the Variance Inflation Factor (VIF; vifstep, usdm R package (Zuur, 2009; Zuur *et al.*, 2010) and pairwise correlations between all potential model predictors through Pearson coefficients (cut-off of $r \leq |0.70|$) **(S3-Table 19)**.

To analyze the relationship between flow intermittence metrics, aquatic hyphomycetes richness and non-hydrological environmental factor, we used linear regression models (LMs) and a multi-model inference approach (Burnham & Anderson, 2002). First, we built 15 separate LMs including the different combinations of flow intermittence metrics and non-hydrological environmental factor as predictors **(Table 8)**. We built these models considering the correlations between the metrics and non-hydrological environmental factor in order to avoid high model collinearity. Second, we ranked the 15 alternative models based on their second-order Akaike

Information Criterion (AICc) for small sample sizes, and retained those with a difference of AICc ≤ 2 with respect to the first ranked model. We also derived total explained variance (R^2) and Akaike weights (w_i) for each model, to provide the explanatory capacity and relative likelihood of each model, respectively. Furthermore, we partitioned the variance explained by each predictor(Hoffman & Schadt, 2016).

To explore if community responses were explained by resistance or resilience mechanisms, we assessed the relationship between beta-diversity components (species turnover and nestedness dissimilarity) and flow-intermittence distance (Euclidean distance) using Mantel tests corrected by spatial autocorrelation through Moran spectral randomization (MSR; Crabot *et al.*, 2019) (999 runs for each test). The MSR correction removed the spurious spatial dependence from our flow-intermittence distance (Euclidean distance), providing a correlation value which reflects the net intermittence importance. We used Baselga's approach (Baselga, 2010) to partition beta-diversity (Sørensen index) changes into species turnover (Simpson index) and nestedness-resultant components using betapart package (Baselga & Orme, 2012).

To analyze the relationships between flow intermittence, richness and organic matter decomposition, we used a Structural Equation Modelling (SEM) approach (Lavaan and LavaanPlot R packets). SEM is a causal inference tool to examine the complex networks settled in natural ecosystems, since several influences and responses can be analyzed simultaneously (Shipley, 2004; Grace, 2006; Grace *et al.*, 2010). We tested our theoretical model, which includes a complete set of direct and indirect causal relationships **(Figure 20)**. The causal meta-model was independently tested for field observations (n = 15). Path coefficients were fitted using the

maximum likelihood algorithm. Overall model fit was assessed through the likelihood chi-square value (χ^2) (Iriondo *et al.*, 2003), Goodness of Fit Index (GFI) and Root Mean Square Error of Approximation (RMSEA). GFI values above 0.9 and RMSEA values below 0.05 indicate a good fit of the model (Gana & Broc, 2019). The proposed model **(Figure 20)** did not show a good fit due to the small number of field observations; to solve this problem, we excluded the direct relationship between flow intermittence and decomposition, and the new model showed an excellent fit.

Microcosm experiment

To evaluate differences in the endpoints tested (leaf decomposition, fungal biomass) in response to flow intermittence and fungal richness, we used linear models and a multi-model inference approach. For each endpoint, we fitted two models including only one predictor (either flow intermittence or fungal richness), one additive model (flow intermittence and fungal richness) and one model including both single terms plus their interaction. Based on their AICc values, we ranked these four models and retained those with a difference of AICc ≤ 2 with respect to the first ranked model, and partitioned explained variance by each predictor. Finally, for models ranked first, we performed post hoc Tukey pairwise comparisons using the multcomp package in R.

For all models, we assessed residuals to verify linear model assumptions of normality and homoscedasticity (Zuur *et al.*, 2010).

All the statistical analysis was performed using R statistical software version 3.4.1 (R Development Core Team, 2011).

RESULTS

Field experiment

Alpha diversity patterns

Of all possible models **(S3-Table 20)**, the one that included total non-flow days (TNF) and the non-hydrological environmental factor (ENV), namely Mod5, best explained the patterns of aquatic hyphomycete species richness **(Table 8)**.

Table 8. Ranking of linear models based of species richness. ENV is the value of the first axis of the PCA (see details in **S3-Environmental characterization**), TNF is total number of non-flow days, NFP is non-flow periods and NF is days of the last non-flow period. Columns represent the predictor effect size of ENV and the flow intermittence metrics. Goodness of fit (R^2) and weight are also shown.

		Flow intermittence metrics						
Mod	ENV	TNF	NFP	NF	R^2	AICc	ΔAICc	weight
5	**3.32**	**-4.89**			**49.7**	**96.4**	**0**	**0.48**
2		-3.37			28.7	98.7	2.28	0.15
3			-2.82		20.02	100.5	4.1	0.06
8	3.70	-5.54	0.81		48.38	100.8	4.39	0.05
9	3.26	-4.82		-0.12	47.56	101	4.66	0.05
4				-2.60	17.02	101.1	4.69	0.05
13		-2.80		-1.62	32.85	101.1	4.73	0.05
14			-2.47	-2.21	30.53	101.7	5.29	0.03
12		-2.61	-1.27		29.7	101.9	5.49	0.03
1	1.09				2.935	103.6	7.2	0.01
6	0.83		-2.74		20.45	103.9	7.57	0.01
7	0.49			-2.49	16.47	104.8	8.4	0.01
15		-1.94	-1.40	-1.69	34.08	105	8.58	0.01
10	0.36		-2.45	-2.13	29.01	106.3	9.89	0.00
11	3.84	-5.73	0.91	0.19	46.06	106.6	10.21	0.00

Fungal richness declined with increasing annual number of non-flow days, and increased with increasing ENV, i.e., an increase in streams with more altitude and, natural land use as well as higher water oxygen concentrations. The percentage of variance explained by the model was 49.7% **(Table 8)**. Intermittence (TNF) was responsible for most of the explained variance, ca. 50.4% of the total variance whereas ENV explained only ca. 3.2%. We should point out that these were individual contributions and no explained variation was shared by ENV and TNF.

Beta diversity patterns

Overall, 92.7% of the overall beta diversity dissimilarity was due to species turnover, while nestedness had a much lower contribution (7.2%) (**S3-Figure 6**). Mantel tests showed that communities that experience different flow-intermittence patterns tend to have higher overall dissimilarity (Mantel r=0.43; p=0.008) and a greater contribution of species turnover (Mantel r=0.42; p=0.016). Contrastingly, the relationship between nestedness-resultant dissimilarity and flow-intermittence distance was weak and non-significant (Mantel r=-0.19; p=0.892).

Effect of flow intermittence and richness on organic matter decomposition

Our SEM results showed that an increase in the total number of non-flow days reduced decomposition indirectly, through a significant decrease of aquatic hyphomycete richness **(Figure 23 and 24)**. In the model, the explained variance reached 30.2% for richness and 42.7% for decomposition. Total non-flow days affected aquatic hyphomycete richness

negatively, and aquatic hyphomycete richness affected decomposition positively.

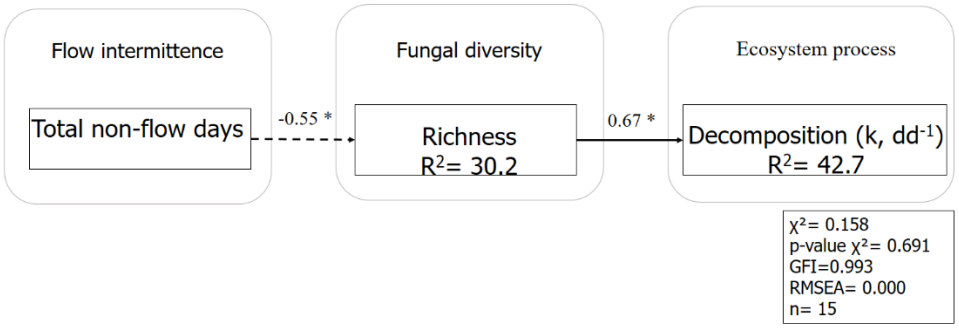


Figure 23. Causal paths of SEM analyses showing the relationships between flow intermittence, aquatic hyphomycete richness and ecosystem function (organic matter decomposition). Solid arrows represent positive relationships and broken arrows represent negative effects (* $p<0.005$). R^2 denotes the proportion of variance explained, and appears below every response variable.

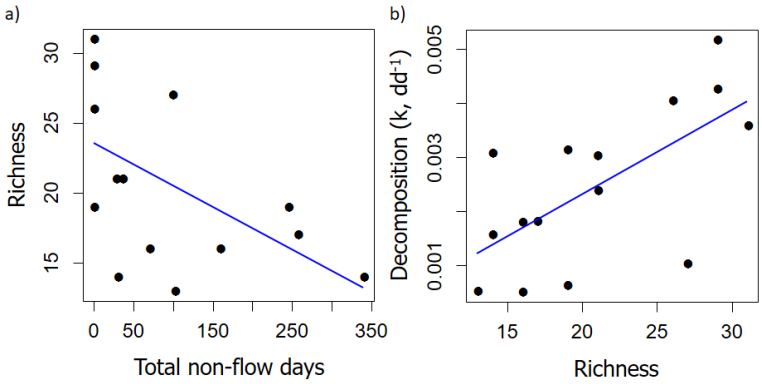


Figure 24. Relationships between: aquatic hyphomycete richness and total non-flow days (a); decomposition and aquatic hyphomycete richness (b). Fitted values are based on a linear regression between the variables showed at each panel.

Microcosm experiment

Leaf litter decomposition and fungal biomass were significantly affected by richness, and the flow intermittence treatments **(Table 9)**.

Leaf litter decomposition was significantly higher in all high richness treatments (mean difference: 0.10; Tukey HSD, p=0.009; **Figure 25a**). Significant differences were also found between permanent and ephemeral treatments (mean difference: 0.12; Tukey HSD, p=0.03). Fungal biomass was significantly higher in the higher richness treatments (mean difference: 0.70; Tukey HSD, p<0.000) **(Figure 25b)**. We also found significant differences between all flow intermittence treatments (mean difference: 2.47; Tukey HSD, p<0.0001 between permanent and ephemeral; and mean difference: 1.82; Tukey HSD, p<0.0001 between intermittent and ephemeral treatments; mean difference: 0.64; and p=0.004 between permanent and intermittent treatment). Fungal biomass was significantly higher at increase richness, under intermittent and ephemeral treatments, but not thepermanent treatment (details of Tukey HSD interaction of richness and treatment in **S3-Table 21**). In addition, aquatic hyphomycetes recovered after the non-flow period in intermittent treatments, as shown by an increase in species richness after rewetting **(Figure 26)**.

Table 9: Results of ANOVAs testing for the effect of fungal richness (Ric) and flow intermittence treatment (Treat) on leaf litter decomposition (i.e., dry mass loss) and fungal biomass (n=30).

Predictors		Leaf litter decomposition			Fungal Biomass		
	df	F	P-value	Explained variance	F	P-value	Explained variance
Ric	1	7.9	**<0.001**	19.2	21.8	**<0.001**	8.2
Treat	2	3.7	**0.03**	18.1	98.9	**<0.001**	72.2
Ric x Treat	2	-	-	-	11.3	**<0.001**	8.5

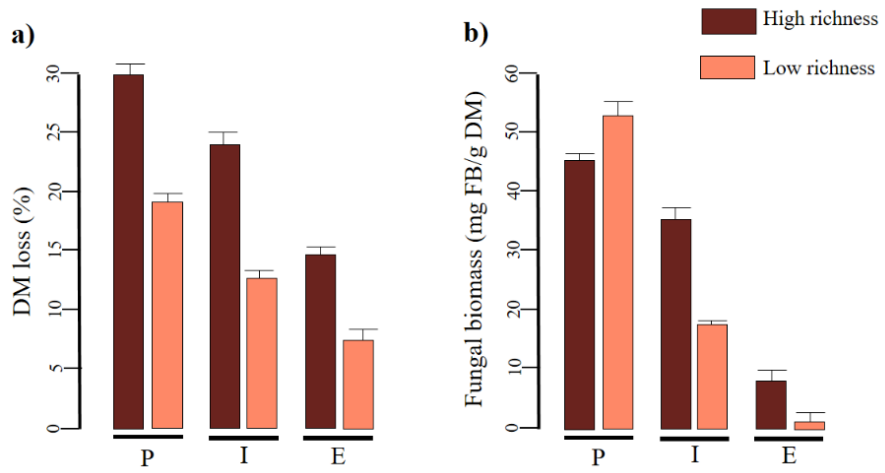


Figure 25. Percentage of dry mass (DM) loss (a), and fungal biomass (b). P is permanent treatment; I is intermittent treatment; and E is ephemeral treatment.

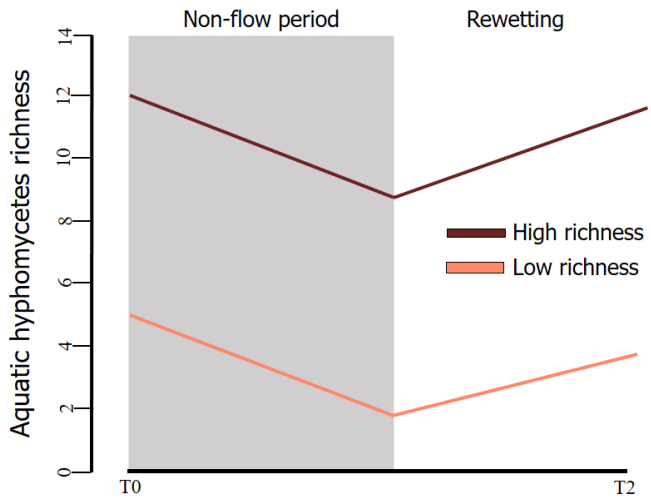


Figure 26. Evolution of aquatic hyphomycete richness in the intermittent treatments (n=3). T0 is the start point and T2 he endpoint of the experiment

Discussion

Our results suggest that longer non-flowing periods lead to reduced aquatic hyphomycete richness, compositional changes (species turnover) and lower rates of organic matter decomposition. Using both observational and experimental approaches, we have shown that aquatic hyphomycetes richness attenuated or mediated the effect of flow intermittence on organic matter decomposition.

In agreement with our hypothesis, flow intermittence was the most important predictor of aquatic hyphomycete biodiversity (alpha and beta diversity). Even when considering background environmental variability and different intermittence metrics, such as the number of non-flow periods or rewetting duration, the total number of non-flow days was the most relevant

variable. Previous manipulative experiments also found that the duration of the non-flow period was the key factor determining microbial activity (Bruder *et al.*, 2011; Gonçalves *et al.*, 2016; Duarte *et al.*, 2017). Overall, the annual hydrological regime seems to explain aquatic hyphomycete species richness better than recent disturbances, because of their capacity to respond quickly as a result of their short life cycles and highly dynamic populations (Bärlocher, 2009; Grimmett et al., 2013). For instance, even some flash storms can rapidly stimulate and restore their activity (Bruder *et al.*, 2011; Foulquier *et al.*, 2015; Gionchetta *et al.*, 2019), upon rewetting, microbial activity, as spore production resumes in just a few hours, leading to a recovery of the species within communities (Bärlocher, 2009) **(Figure 26).** Regarding non-hydrological environmental variability, previous studies revealed that the growth and development of microbes is highly sensitive to changes in water temperature, dissolved nutrients and oxygen, because aquatic hyphomycetes are intimately associated with their substrate (Chauvet *et al.*, 2016). However, in our study, this factor was of minor importance because we aimed to minimize non-hydrological variability.

Despite their high substrate-specificity, aquatic hyphomycetes are ubiquitous (Bärlocher, 2016; Selosse et al., 2008). Habitat filtering can be the main process that explain the composition of local assemblies of aquatic hyphomycetes. Indeed, we found a decline in the number of species along the intermittence gradient, as observed for other aquatic groups (Soria *et al.*, 2017) and other stressors. Furthermore, non-flow periods inhibit spore production (Bärlocher et al., 2013; Canhoto et al., 2016; Medeiros et al., 2009), and when spore production is inhibited, dispersal will be limited. Thus strong habitat filtering could sort species in relation to their tolerance to desiccation stress (Odum, 1985; Gutiérrez-Cánovas *et al.*, 2013). Moreover, in line with our prediction, we have demonstrated that compositional

changes arise from species replacement, revealing that aquatic hyphomycetes have a strong affinity for different intermittence regimes and that resistance adaptation mechanisms could mediate this response, rather than resilience (Datry et al., 2014). Our turnover patterns differ from those focused on aquatic macroinvertebrate in IRES, where nestedness and resilience responses were the main mechanisms explaining compositional changes over the intermittence gradient (Arscott et al., 2010; Corti & Drummond, 2011; Datry, 2012). This disparity of results could be related to the higher dispersal capacity of macroinvertebrates and their terrestrial and aquatic life cycles, especially for strong flyers or those that can disperse by wind, which could enhance rapid response to drying conditions and colonization when habitat conditions are favourable (Bogan *et al.*, 2013). The fact that aquatic hyphomycetes spend their entire life cycle under-water reinforces the idea that resistance and specialization mechanisms should explain compositional changes. Such specialization could be related to physiological or functional adaptations that have emerged over evolutionary time in response to recurrent and predictable patterns of desiccation (Belliveau & Bärlocher, 2005). These traits probably arose from multiple convergent evolutionary pathways, by secondary adaptation to aquatic life, as recently confirmed by molecular markers (Belliveau & Bärlocher, 2005; Baschien *et al.*, 2006); despite their physiological and functional adaptations having been overlooked.

In relation to resistance mechanisms, some studies have shown the importance of refugial habitats as microbial resistance strategies; for instance, it has been demonstrated that bacterial communities can survive in the subsurface zone during long-term non-flow periods thanks to episodic flash storms that suddenly increase the water content in the sediment (Gionchetta *et al.*, 2019, 2020; Arias-Real *et al.*, 2020). Such survival

capacity has implications for the maintenance of nutrient cycling and organic matter decomposition during the non-flow period (Marxen *et al.*, 2010; Pohlon *et al.*, 2013; Harjung *et al.*, 2019). Therefore, this zone could also be a potential source of fungal propagules when surface flow ceases, although conditions for their developed are not optimal, particularly for species with filiform spores such as *Flagellospora curvula,* which will be able to disperse in interstitial water in contrast to those with compact (e.g., *Heliscus lugdunensis*) or branched/tetraradiate (e.g., *Lemoniera aquatica*) morphologies (Cornut *et al.*, 2014). For example, Ghate and Sridhar (Ghate & Sridhar, 2015) observed the presence of *Anguillospora longissima* and *Flagellospora curvula* in IRES sediment in Southwestern India. Another potential refuge could be leaf packs. Some authors have suggested that some aquatic hyphomycetes could have a dual life cycle, including a terrestrial phase as an endophyte with some dormant structures, which could be seen as desiccation-resistance life strategy adaptations that allow survival in IRES (Bärlocher, 2009; Selosse et al., 2008).

Therefore, exploring beta diversity patterns is a useful approach to disentangle the roles of community resistance and resilience mechanisms in IRES, for a group for whose functional traits are largely unknown. This study is one of few to analyse alpha and beta community changes of aquatic hyphomycetes over flow intermittence gradients, and to explore how aquatic hyphomycete biodiversity drives organic matter decomposition in IRES (see for example: Artigas *et al.*, 2008; Foulquier *et al.*, 2015; Ghate & Sridhar, 2015; Mora-Gómez *et al.*, 2015). Overall, in a context of anthropogenic global change and biodiversity reduction, our results suggest that the loss of microbial diversity will threaten the functioning of river ecosystem. To further explore this idea, we suggest that additional studies should be conducted

using molecular targets of specific fungal traits, both response and effect traits.

In addition, the lower rates of organic matter decomposition in response to intermittence and the reduction in aquatic hyphomycete richness could arise from a decrease in species decomposing efficiency and mechanisms of complementarity. First, stress specialists could decompose OM less efficiently as a result of a expending more energy on mechanisms to cope with desiccation (see examples of microbes in hot, acidic or saline systems: Medeiros *et al.*, 2009; Azevedo & Cássio, 2010; Canhoto *et al.*, 2016, 2017). Second, greater diversity of species could be enhanced greater functions and complementarity, which would raise decomposition rates (Gessner *et al.*, 2010). For example, different species of aquatic hyphomycetes could possess complementary enzymes able to degrade a range of organic matter, or maybe species with complementary enzymes could present different activity patterns in response to environmental conditions (Gessner *et al.*, 2007), or even some species could facilitate the penetration of others and all of them together may increase decomposition rates. Consequently, when flow intermittence increases, some of these mechanisms could be affected and thus alter leaf litter decomposition. We hope this finding stimulates future research to explore aquatic hyphomycete functional diversity, and the mechanisms underlying the effects of biodiversity on plant litter decomposition.

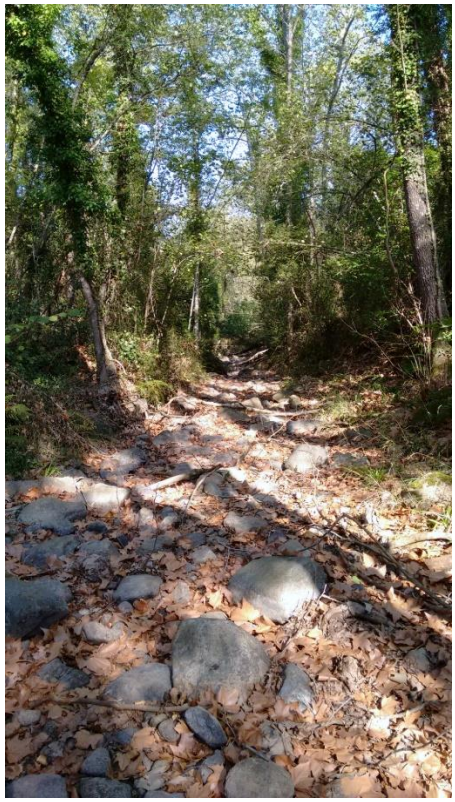
Taken together, our results demonstrate the importance of aquatic hyphomycetes in sustaining ecosystem functions in rivers and how flow intermittency can compromise organic matter decomposition through alterations in their biodiversity. Although habitat filtering could render less diverse habitats in terms of local richness, the high degree of specialization

of aquatic hyphomycetes could provide additional support to the decomposition process. Our results suggest that anthropogenic pressures, such as climate change or water abstraction, could jeopardize organic matter turnover at the global scale if fungal richness is affected. Our study helps to understand ecosystem functioning better and also the consequences of biodiversity loss in IRES.

Acknowledgements

We gratefully acknowledge the field survey assistance of Verónica Granados.

Microcosms experiment.



Example of sampling sites.

CHAPTER 4

Quality and quantity of leaf litter: both are important for feeding preferences and growth of an aquatic shredder

Quality and quantity of leaf litter: both are important for feeding preferences and growth of an aquatic shredder.

Rebeca Arias-Real[1], Margarita Menéndez[1], Meritxell Abril[2], Francesc Oliva[3] and Isabel Muñoz[1]

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain.

[2] BETA Technological Centre, University of Vic-Central University of Catalonia, Vic, Spain.

[3] Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain.

Published in PLoS ONE.

https://doi.org/10.1371/journal.pone.0208272

Abstract

The study of leaf litter as a resource for shredders has emerged as a key topic in trophic links in ecology. However, thus far, most studies have emphasized the leaf quality as one of the main determinants of shredder behaviour and growth without simultaneously considering the leaf quantity availability. Nevertheless, the combined effects of leaf quantity and quality on shredder behaviour and growth is particularly crucial to further understand how ecosystem functioning may respond to the increasing flow intermittency due to climate change. In this study, we explore how changes in the leaf litter quality and quantity influence the feeding preferences and growth of an invertebrate shredder (*Potamophylax latipennis*). To do so, we used black poplar leaves conditioned in two streams with different flow regimens as a food resource. Afterwards, using a microcosm approach, we offered leaf discs that varied in terms of leaf quantity and quality to *P. latipennis*. Our results showed that flow intermittency had a negative effect on the quality of the food resource, and a lower quality had a negative effect on the consumption and growth rates of *P. latipennis.* Furthermore, we found that *P. latipennis* fed selectively on higher quality leaves even though the availability (quantity) of this resource was lower. In the context of climate change, with higher aridity/drier conditions/scenarios, our findings suggest that a decrease in the availability (quantity) of high-quality resources could potentially threaten links in global fluvial food webs and thus threaten ecosystem functioning.

Introduction

Rivers that naturally and periodically cease to flow in time and/or space are termed intermittent rivers (IRs) and are recognized as the most common fluvial ecosystem around the world (Datry *et al.*, 2014b). The seasonal flow variability in IRs is the most important factor that determines their functioning; for instance, this variability determines the nutrient dynamics or hydrological connectivity essential for community dispersion (Larned *et al.*, 2010).

As a result of the increasing aridification caused by climate change, many streams are expected to become IRs, experiencing greater variability in their water flow, which may eventually lead to complete flow disruption (Martínez *et al.*, 2015). Despite the importance and increasing abundance of IRs, the effects of changes in water flow on biodiversity and ecosystem functioning are still largely unknown (Leigh *et al.*, 2016).

Organic matter decomposition is a key ecosystem process that influences the cycling of nutrients and energy flow to higher trophic levels (Gonçalves *et al.*, 2014; Abril *et al.*, 2016) . Microbial decomposers (fungal and bacterial communities) and invertebrate shredders are the biological drivers of organic matter decomposition. Among the microbial decomposers, aquatic hyphomycetes (fungal community) are considered the first colonizers and main drivers of microbial decomposition in its first stages (Gessner *et al.*, 2010), which constitutes an important trophic link between leaf litter and shredders (Kuehn, 2016). Aquatic hyphomycetes improve the palatability and nutritional quality (increasing the proteins, lipids and carbohydrates due to the characteristics of the fungi themselves) of leaf litter by transforming recalcitrant polymers into more labile molecules via their enzyme capabilities

(Bärlocher, 2016; Mas-Martí et al., 2015) and increasing the nitrogen and phosphorus concentrations of leaf litter via the accumulation of fungal mycelia (Chung & Suberkropp, 2009; Grimmett *et al.*, 2013). This leaf transformation is crucial for shredders, which need a critical amount and balance of inorganic and organic elements for growth, reproduction and maintenance (Mas-Martí *et al.*, 2015). Moreover, the shredders cannot synthesize some essential components (e.g., essential fatty acids) and must therefore acquire them from their diet (Cargill *et al.*, 1985b). Consequently, shredders tend to consume the most optimal resource, which is the most energetic and nutrient-rich food available.

Generally, flow reduction affects the communities of aquatic hyphomycetes, which are particularly vulnerable to desiccation stress, especially those that are not adapted to flow reduction (Costantini & Rossi, 2010). Some studies have shown that during flow reduction, the communities of aquatic hyphomycetes can experience a shift in fungal richness and composition and alterations in their enzymatic activity, such as summer drying conditions inhibiting lignocellulolytic enzyme activities (Bärlocher, 2016; Gonçalves et al., 2016; Mora-Gómez et al., 2016). These changes in the communities of aquatic hyphomycetes coupled to changes in the abiotic conditions of streams under flow reduction (e.g., decreasing the dissolved oxygen content and increasing the water temperature and conductivity) are expected to affect organic matter decomposition and the feeding links to higher trophic levels (Covich *et al.*, 1999; Dieter *et al.*, 2013).

In addition, flow reduction also affects the riparian vegetation, causing early leaf abscission (Sanpera-Calbet *et al.*, 2017b). This may lead to temporal and spatial changes in this basal resource for aquatic hyphomycetes and shredders. Despite its potential implications for the organic matter cycle, the

effects of decreasing the availability of high-quality resources, such as leaf litter, on consumers is still poorly studied.

To date, studies have focused on leaf quality to explain invertebrate shredder behaviour and growth (Richardson *et al.*, 2010; Marcarelli *et al.*, 2011; Kaspari *et al.*, 2012). For example, Frost et al. (Frost *et al.*, 2005, 2006) have shown that shredders compensated for nutrient limitations by increasing feeding rates or selectively feeding on resources with more nutritious properties. Nevertheless, little is known regarding the effect of combined changes in leaf quantity and quality on the feeding preferences of invertebrate shredders and their growth. This knowledge is particularly crucial for understanding how biodiversity (e.g., aquatic hyphomycetes community) and ecosystem functioning (e.g., organic matter decomposition and its implications on stream food webs (Marcarelli *et al.*, 2011) may respond to ongoing effects of climate change.

In line with this information, we address this knowledge gap herein by exploring how changes in both the leaf litter quality and quantity affect or determine the feeding preferences and growth of an invertebrate shredder using field and microcosm approaches. To do so, we first assessed the influence of flow intermittency on the leaf litter quality (thought fungal biomass, C:N ratios and total lipid content) and the composition of the associated community of aquatic hyphomycetes. We expected that under flowing conditions (a permanent stream) the leaf litter would be of better quality (lower C:N ratios and higher fungal biomass and lipid content) than that from an IRs. Second, we explored the joint effects of leaf litter (food resource) quality and quantity on the consumption rates and growth of a shredder using microcosms. We expected that better quality food resources and availability would be correlated with higher consumption rates and

growth of the shredder. Finally, we quantified the feeding preferences of the shredder, expecting that the quality rather the quantity of the resource would be more important. Therefore, even if the shredder has a larger quantity of poor-quality resources, they will actively select the best quality food.

Materials and methods

Leaf litter and fungal assemblages

We used black poplar leaves (*Populus nigra* L.*)* conditioned in two different streams as a food resource. We selected one permanent stream (Arbucies in the Tordera Basin, N 41º 823133 E 2º 452826; hereafter termed "A") and one intermittent stream (Llobina in the Besos Basin, N 41º 46.011 E 2º 16.104; hereafter termed "B") in Catalonia, Spain. Both streams are third-order streams, meaning that they have different flow regimes but similar water physicochemical characteristics (Menéndez *et al.*, 2012). Furthermore, both streams have the same geology (siliceous bedrock) and poplar (*Populus nigra* L.), alder (*Alnus glutinosa* (L.) Gaertner) and evergreen oak (*Quercus ilex* L.O) are the dominant riparian vegetation. In addition, previous studies have indicated differences in the biodiversity of aquatic hyphomycetes between these streams (Menéndez *et al.*, 2013) , with lower diversity in the Llobina stream (B).

To characterize the stream hydrology, in February 2016, we placed Leveloggers (Solinst Levelogger Edge, full-scale reading precision of 0.05%) on the streambed for water level and temperature recordings as a proxy to measure the presence or absence of flow. The Leveloggers were recorded at

hourly intervals for one year (from February 2016 until January 2017). The recorded data were corrected with barometric pressure variations using data from Barologgers (Solinst Barologger, full-scale reading precision of 0.05%). We installed the Barologgers at each site in the riparian area to measure the atmospheric pressure changes. In addition, throughout this year, we collected samples two times (in October 2016 and January 2017) to analyse the water concentrations of nutrients (nitrate, nitrite, ammonium and soluble reactive phosphorous; SRP) from both streams.

Black poplar leaves were collected freshly abscised in autumn 2016 from the riparian area of A stream and dried at room temperature until needed. The dried leaf sets were inserted into mesh bags (0.5 mm mesh size and with approximately 10-12 leaves) and transferred to the streams (23 mesh bags in each stream) along a 100 m reach.

As previous studies demonstrated that the conditioning process follows a unimodal pattern in which the palatability of leaves increases to a maximum within 2 to 6 weeks and then declines, we removed the leaf mesh bags from each stream after 20 days of conditioning (Arsuffi & Suberkropp, 1984; Suberkropp & Arsuffi, 1984). Afterwards, the mesh bags were transferred to the laboratory and 945 leaf discs of 16 mm diameter were obtained with a cork borer, avoiding veins. We repeated this process twice (with a week in between) to supply similar food conditions to the shredder (see below).

Leaf litter quality after 14 days of conditioning

We used a set of leaf discs from each stream to characterize the initial communities of aquatic hyphomycetes. To induce conidial release from the hyphomycetes, five leaf discs with six replicates per stream were placed into 10 Erlenmeyer flasks with 60 ml of dechlorinated water at 14ºC under 12 h

light: 12 h dark conditions for 48 h. We aerated the Erlenmeyer flasks from the bottom by a continuous airflow to create turbulence that kept the leaf discs in continuous motion (Dang *et al.*, 2005). To determine the initial communities of aquatic hyphomycetes in the leaf discs, we filtered the suspensions of spores through 5-µm-pore size membrane filters (Cellulose Nitrate Membrane Filters, Whatman) and stained the filters with one drop of Trypan Blue solution containing lactic acid. For all the samples, we filtered the same volume (5 ml) of the suspensions of spores. Then, we used fields for identify and count the spores. First, we scanned the surface of the filter under the light microscope (400x), then we counted and identified all conidia and if they were very numerous, we counted the conidia in 20-30 randomly chosen microscope fields. We expressed the result as number of conidia per mL. Furthermore, based on these results we created a presence or absence conidia matrix (Gönczöl *et al.*, 2001).

The ergosterol concentration in the leaf discs was determined as a proxy for fungal biomass (Gessner, 2020). Five frozen leaf discs per five replicates per stream were lyophilized and weighed to determine the dry mass. We performed the lipid extraction and saponification using 0.14 M KOH methanol (8 g L^{-1}) at 80 °C for 30 min in a shaking water bath. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp., Milford, MA, USA), and ergosterol was eluted using isopropanol. We used high pressure liquid chromatography (HPLC) to detect and quantify the ergosterol by measuring the absorbance at 282 nm. We used a Jasco HPLC system (USA) equipped with a Gemini-NX 5 µm C18 250 × 4.6 mm column (Phenomenex, UK). The mobile phase was 100% methanol, and the flow rate was set to 1.2 ml min^{-1}. Finally, we converted the ergosterol into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium (Gessner & Chauvet, 1993).

We expressed the results in mg of fungal biomass per gram of dry mass leaf litter.

To determine the total lipid content in the leaf, we lyophilized five frozen leaf discs per five replicates per stream and homogenized them with an ultrasonic homogenizer (200 W, 24 kHz; Hielscher Ultrasonics GmbH, Teltow, Germany). We performed the lipid extraction with a monophasic solution of chloroform and methanol (2:1, v/v). Then, using a biphasic solution (chloroform and distilled water), we separated the phases, and after one night at 50ºC, we analysed the total lipid content using the colorimetric sulpho-phospho-vanillin method (Zöllner & Kirsch, 1962). We expressed the results as the percentage of total lipid.

Finally, another set of five leaf discs per five replicates from each stream was dried and ground into a fine powder to analyse the nitrogen (N) and carbon (C) concentrations using a Thermo Element Analyzer 1108 (Thermo Scientific, Milan, Italy). We expressed the results in terms of C:N molar ratios.

Shredder

We collected individuals of *Potamophylax latipennis* (Trichoptera: F. Limnephilidae, Curtis, 1834) in stream A in February 2017 and transported them to the laboratory in plastic containers containing stream water and sand. To acclimatize the shredders to the laboratory conditions, they were maintained in declorinated water with food (leaves from the river) provided ad libitum for three days before starting the experiment. The temperature was maintained at 14ºC. On the third day, the shredders were starved, which allowed evacuation of their gut contents. Once the shredders were acclimatized, we sorted 86 shredders with similar size. To calculate the average initial larval weight, average initial head width and the average initial

lipid content, we separated thirty-six individuals we measured and frozen them at -80ºC, lyophilized, and weighed (initial head width=1.79 ± 0.03 mm; initial larvae dry mass= 0.031 ± 0.07 mg, n=36).

Microcosm setup

We allocated the shredders individually into fifty glass microcosms (8.5 cm diameter and 8.3 cm height) with 60 ml of dechlorinated water. We added 12 leaf discs in each microcosm according to five different treatments: Treatment 1 (t1), ten microcosms with leaf discs from only stream A; treatment 2 (t2), ten microcosms with leaf discs from stream B; treatment 3 (t3), ten microcosms with equal proportions of leaf discs from streams A and B; treatment 4 (t4), ten microcosms with 75% of the leaf discs from stream A and 25% of the leaf discs from stream B; treatment 5 (t5), ten microcosms with 25% of the leaf discs from stream A and 75% of the leaf discs from stream B. In mixture treatments (from t3 to t5), discs from each type of leaves (A or B) were marked with colour pins. In addition, twenty microcosms containing leaf discs (ten with leaf discs from only stream A and ten with leaf discs from stream B) were maintained without shredders to serve as the controls for the loss of leaf not attributable to consumption **(Figure 27)**.

We provided each microcosm with ash sand from the same riverbed (previously burnt at 450ºC for 4 h) to allow larvae to build their cases. We aerated the microcosms by a continuous airflow under 12 h light: 12 h dark photoperiod conditions at 14ºC in incubator rooms. The experiment lasted 2 weeks. Every three days during the experiment, we controlled the NH_4 concentration of each microcosm (Tetra Test NH_3/NH_4, Tetra GmbH, Germany) and the water level. If the water level was below 60 ml, we added dechlorinated water until that level was reached. At the end of the first week,

we replaced the water and leaf discs from those colonized one week later in the streams. All leaf discs were kept frozen at -80ºC until analysis.

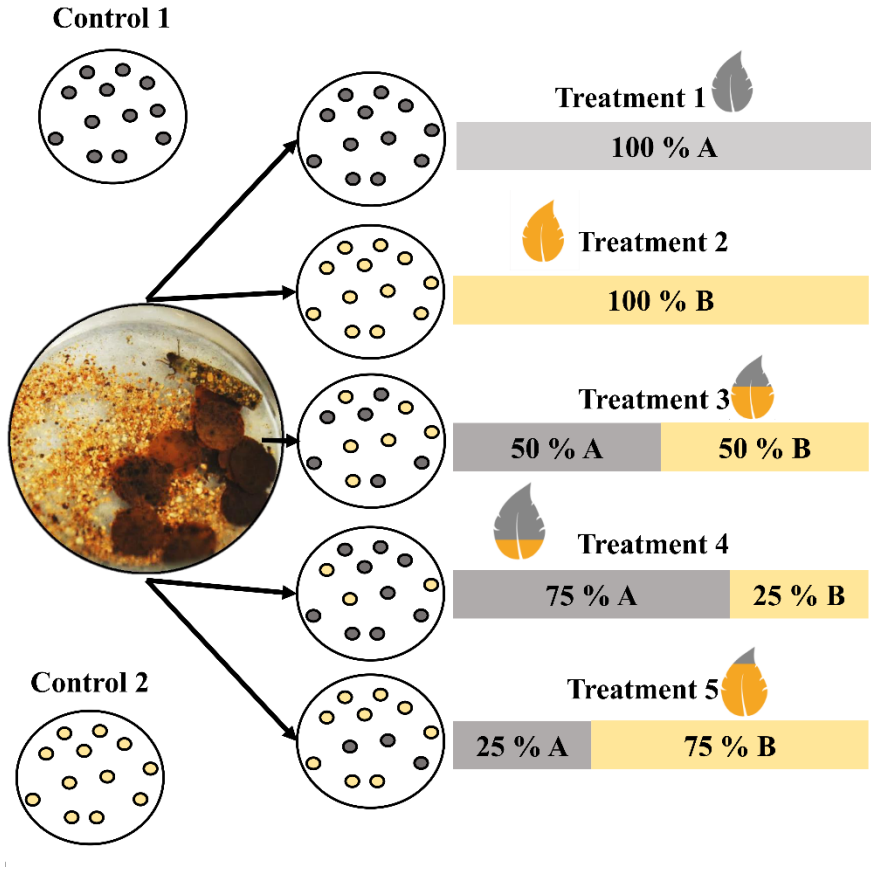


Figure 27. Experimental design. A and B refer to the leaf discs from the permanent and intermittent streams, respectively. Each microcosm contained individual shredders and 12 leaf discs. Ten microcosms were used per treatment.

Shredder consumption and growth

We determined the shredder consumption (C, mg) at the end of the experiment in each treatment as follows:

$$C = \sum_{k=1}^{2}(Li - Lf) \quad (1)$$

where *k* is the number of weeks of the experiment, and *Li* and *Lf* are the initial and final dry masses (hereafter DM, mg) of leaf discs for each week of the experiment, respectively, corrected by the DM leaf loss in the control microcosms without shredders of the respective treatments.

We calculated the relative consumption rate per larvae (RCR, mg leaf DM mg $^{-1}$ larval DM day $^{-1}$) as follows:

$$RCR = \frac{C/T}{w} \quad (2)$$

where *T* is the time for the entire feeding period (14 days) and *w* is the average of the larval DM (mg) at the beginning and end of the experiment.

We calculated the instantaneous growth rate (IGR, mm d^{-1}) and the relative growth rate (RGR, mm mm $^{-1}$ d^{-1}) using the head width (HW) of the larvae (Chung & Suberkropp, 2009) as follows:

$$IGR = \frac{\ln(HWf) - \ln(HWi)}{T} = \frac{\ln(\frac{HWf}{HWi})}{T} \quad (3)$$

and

$$RGR = \frac{HWf - HWi}{(HWf * T)} \quad (4)$$

where *HWf* and *HWi* are the final and initial head width (mm), respectively and *T* is the time for the entire feeding period (14 days).

We measured the metabolism via the oxygen consumption for nearly 10 min and corrected the measurement by the individual dry weight (mg O_2 L^{-1} g DM^{-1} min^{-1}). Measurements were made with an optical oxygen microsensor adapted to a 20 ml glass vial (Fibox 4 PreSens, Regensburg, Germany) filled with the oxygen-saturated water in which the shredder had been introduced. The oxygen concentration was recorded every 5 seconds for 10 min (Warkentin *et al.*, 2007).

Finally, as a proxy for their body condition, we analysed the total lipid content of each shredder, expressed as a percentage of invertebrate DM. At the end of the experiment, each individual was frozen separately at -80ºC. The protocol was similar to that for the leaves. We quantified the lipid content by spectrophotometry after digestion with H_2SO_4 (100ºC) and comparison against a cholesterol standard (Mas-Martí *et al.*, 2015).

Data analysis

To characterize the stream hydrology in the intermittent stream, we used the daily variation of the streambed temperature corrected for the barometric pressure and air temperature. This daily variation was determined as the difference between the maximum and minimum temperature per day and the daily higher rate of change per hour. We performed a fifth-order moving average to smooth daily differences. To test the differences in water temperature, water concentrations of nutrients (nitrate, nitrite, ammonium and SRP) from both streams, we performed a two samples t-test at the 95% confidence interval level.

To analyse the effects of flow regime on the leaf litter quality (chemical composition of leaf litter) and richness of aquatic hyphomycetes between streams A and B, we performed two sample t-tests at the 95% confidence

interval level. To test differences between the composition of the conidia produced by the aquatic hyphomycetes colonizing the leaf litter from streams A and B, we used a multivariate generalized linear model (MANYGLM model, mvabund R package) due to it is a flexible and powerful framework for analysing abundance data and show a better power properties than distance-based methods (Wang *et al.*, 2012). Indicator taxa were defined for each stream class (A and B) using the indicator species analysis (IndVal) of Dufrene and Legendre (Dufrene & Legendre, 2011). This analysis generates an indicator value index (IV) for each species (based on the presence or absence of a spore matrix) and stream class. The indicator calculation is based on the specificity (maximum when the species occurs in only one stream) and fidelity (maximum when the species is present in both streams). To perform the tests, we used the packages vegan, mvabund, labdsv and ade4 in R.

To evaluate the differences between treatments in the endpoints measured in the larvae, we first checked the outliers of the data, the variable distributions (skewness) and the assumption of normality (Bartlett and Shapiro test). For variables that did not fulfil the assumptions of normality, we transformed the original data using a square root transformation for RCR and total lipid and a $\log_{10}$ (x+1) transformation for RGR and IGR. We performed one-way ANOVA using the treatment as the fixed effects factor with the car and sandwich packages in R. We validated the model visually by assessing the distribution of residuals for normality and homoscedasticity (Zuur *et al.*, 2010). When the null hypothesis was rejected, we performed post hoc Tukey pairwise comparisons using the multcomp package in R.

Finally, to analyse the effect of leaf quantity on feeding preferences of *P. latipennis,* we performed a one sample t-test for the three-mixture treatment

to compare the observed consumption of A leaf discs to the total consumption and the expected value, considering the last one as the real proportion of A leaf discs at each treatment (50%, 75% and 25%).

All the statistical analyses were performed using the R statistical software version 3.4.1 (R Development Core Team, 2011), with the significance level set at $p < 0.05$ for all tests. The datasets used in this study are available in Supporting Information **S4 Table 22**.

Results

The intermittent stream presented a summer drought with 46 dry days and an average temperature of 10.06ºC (± 1.99), whereas the permanent stream presented an average temperature of 10.70 (± 1.90) (differences were not significant, t-test, t_6= -0.34, p-value= 0.741). The intermittent stream SRP was 0.008 ppm (± 0.001), and the permanent stream SRP was 0.013 ppm (± 0.002) (t-test, t_6= -0.94, p-value= 0.367). The total dissolved inorganic nitrogen (DIN = nitrite + nitrate + ammonia) concentration was 0.24 (± 0.07) in the intermittent stream and 0.41 ppm (± 0.13) in the permanent stream (t-test, t_6= -1.92, p-value= 0.086).

Effects of flow regime on the leaf litter quality

The flow regimen significantly affected the quality of the leaf litter after 20 days of conditioning in streams A and B. The quality differed on lipid content, fungal biomass and aquatic hyphomycetes richness **(Table 10)** being higher for the leaf litter conditioning in stream A.

Table 10. Means ± SEM of the initial chemical leaf litter composition (n=5) and aquatic hyphomycetes richness (n=6) and t-tests results for the quality of the leaves in both treatments (A and B).

	Mean (± SEM)		Statistics	
Variable	Permanent (A)	Intermittent (B)	t_8	p-value
Total lipid (%)	4.13 (± 0.07)	3.36 (± 0.02)	9.45	**<0.001**
C:N	63.5 (± 2.9)	51.2 (± 1.5)	2.42	0.051
Fungal Biomass (mg FB/g DM)	33.71 (± 4.02)	17.68 (± 5.09)	4.30	**0.004**
Aquatic hyphomycetes richness	12 (± 0.4)	5 (± 0.6)	8.87	**<0.001**

The structure of the species in the initial aquatic hyphomycetes communities associated with the leaf litter in streams A and B were significantly different (MANYGLM, p=0.001). IndVal analysis revealed that the species *Heliscus submersus, Alatospora acuminta, Tetrachaetun elegans* and *Lemonniera aquatica* were significantly different, and the species *Fusarium* sp. and *Articulospora tetracladia* were marginally significantly different between the streams. Moreover, *Cilindrocarpon* sp.*, Alatospora acuminata, Tetracladium setigerum, Tetracladium marchalianum, Anguillospora longuissima, Tricladium chaetocladium, Lemonniera aquatica* and *Clavariopsis aquatica* appeared in only stream A, whereas *Dendrospora* sp. appeared in only stream B **(Table 11)**. However, MANYGLM analysis also revealed that the dominant species in both streams *Flagellospora curvula.*

Table 11. Results of the indicator species analysis (IndVal), maximum IV significance (IV is the individual value), associated stream class for each species and the frequency of appearance (A and B, permanent and intermittent streams).

Species	Class	IV	p-value	A	B
Flagellospora curvula	1	0.500	1	100	100
Heliscus lugdunensis	1	0.600	0.461	100	66.7
Heliscus submersus	1	0.750	**0.012**	100	16.6
Tetrachaetum elegans	1	0.857	**0.024**	100	16.67
Fusarium sp.	2	0.694	0.062	16.6	83.3
Cylindrocarpon sp.	1	0.667	0.075	66.6	0
Alatospora acuminata	1	1.000	**0.007**	100	0
Articulospora tetracladia	1	0.694	0.074	83.3	16.6
Lemonniera pseudofloscula	1	0.521	0.567	83.3	50
Lemonniera aquatica	1	0.667	**0.049**	66.6	0
Dendrospora sp.	2	0.167	1	0	16.6
Tetracladium setigerum	1	0.167	1	16.6	0
Tetracladium marchalianum	1	0.500	0.205	50	0
Anguillospora longissima	1	0.333	0.453	33.3	0
Tricladium chaetocladium	1	0.167	1	16.6	0
Clavariopsis aquatica	1	0.333	0.446	33.3	0

Effects of leaf litter quality and quantity on *P. latipennis*

Differences in the leaf litter quality between streams A and B significantly affected the consumption and growth of *P. latipennis* **(Table 12)**.

Table 12. Results of one-way ANOVA (factor treatment) of the effects of leaf quality on consumer consumption and growth. N=50 (10 replicates per treatment) except for oxygen consumption, where n=15 (three replicates per treatment).

Variable	Treatment		
	df	F	P
Total Consumption (mg leaf DM)	4	4.481	**0.004**
RCR (mg leaf DM mg larval DM $^{-1}$ day $^{-1}$)	4	4.317	**0.005**
IGR (mm d^{-1})	4	5.622	**0.001**
RGR (mm mm^{-1} d^{-1})	4	5.074	**0.002**
Total Lipids (%)	4	2.877	**0.035**
Oxygen Consumption (mg L^{-1} $mgDM^{-1}$ s^{-1})	4	3.926	**0.041**

Total consumption was significantly higher in treatments with a higher proportion of A leaf discs, i.e., t1 and t4 treatments with 100% and 75% of A leaf discs, respectively. Post hoc comparisons also showed significant differences (Tukey HSD, $p<0.05$) between treatments t1 and t4 and treatments with a lower proportion of A leaf discs, i.e., t3 and t5 treatments, with 50% and 25% of A leaf discs, respectively **(Figure 28a)**. The RCR was also significantly lower in treatment t3 (50% of A leaf discs) than in the t1 and t4 treatments (Tukey HSD, $p<0.05$; Fig 2B).

The IGR based on head larvae width was 0.019 mm ± 0.001 per day for t1 **(Figure 28c)**, whereas for the rest of the treatments, the head width showed lower IGRs (mean ± SEM, t2= 0.005 ± 0.004, t3= -0.000 ± 0.003, t4= 0.005 ± 0.001 and t5= 0.007 ± 0.001), and significant differences were found between treatments t1 and the others (Tukey HSD, $p<0.05$). The RGR **(Figure 28d)** were significantly different between t1 and t2 (Tukey HSD

$p<0.05$). Therefore, when the larvae were fed with leaf discs of higher quality (t1, 100% A leaf discs), their relative growth was higher (mean ± SEM, t1=0.017 ± 0.001, t2=0.004 ± 0.001), and even in the RCR, no significant differences were observed **(Figure 28b)**. Moreover, there were also significant differences between t1 and t3, and surprisingly, between t1 and t4 (Tukey HSD $p<0.001$ and $p= 0.010$, respectively). The t4 treatment showed higher consumption but lower growth. Larvae of this treatment showed a greater proportion of lipids than t1 (Tukey HSD, $p=0.019$) **(Figure 28e)**.

The metabolism of the shredder larvae was significantly higher in t2 (100% B leaf discs) than in the other treatments (Tukey HSD, $p<0.005$) except for t5, in which 75% of the leaves were from B (mean ± SEM, t1= 0.04 ± 0.01; t2= 0.09 ± 0.01; t3= 0.03 ± 0.01; t4= 0.03 ± 0.02 and t5= 0.06 ± 0.01) **(Figure 28f)**. No mortality was observed during the experiment.

When leaf discs from stream A and B were offered simultaneously in different quantities to the shredders (mixture treatments: t3, t4 and t5), the shredders showed a tendency to select leaf discs from A (t-test, $t_{14} = 2.184$, $p = 0.047$).

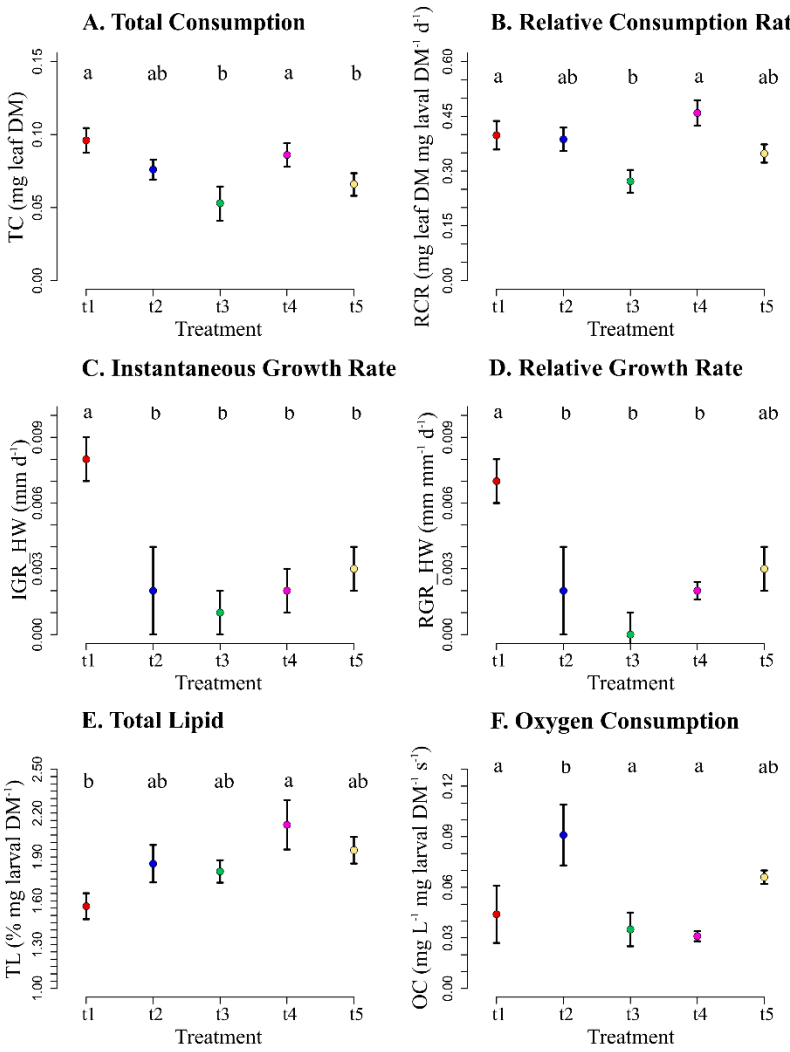


Figure 28. Shredder consumption and growth. Total consumption (A), relative consumption rate (RCR) (B); instantaneous growth rate of the head width (IGR) (C); relative growth rate of the head width (RGR) (D); total lipid content (E) and, oxygen consumption (F) of *P. latipennis*, where t1= 100% A, t2= 100% B, t3= 50% A and 50% B, t4= 75% A and 25% B and t5= 25% A and 75% B. The different letters indicate significant differences (Tukey HSD post hoc test, $p< 0.05$) among treatments for each variable.

The percentages of the quantity of A leaf discs consumed regarding the consumption expected at each treatment according to the quantity of each type of leaf disc were mostly greater than the expected consumption at each treatment **(Figure 29)**. In t3, the larvae consumed 65% of A leaf discs, whereas the expected amount was 50% (15% more than expected), while in t4, larvae consumed 77% and 75% was expected (2% more than expected), and finally, the t5 larvae consumed almost all the A leaf discs (27% more than expected). When A leaf discs were offered below 50% (treatment 5), the shredder fed selectively A leaf discs (t-test, t_5=2.5379, p=0.042).

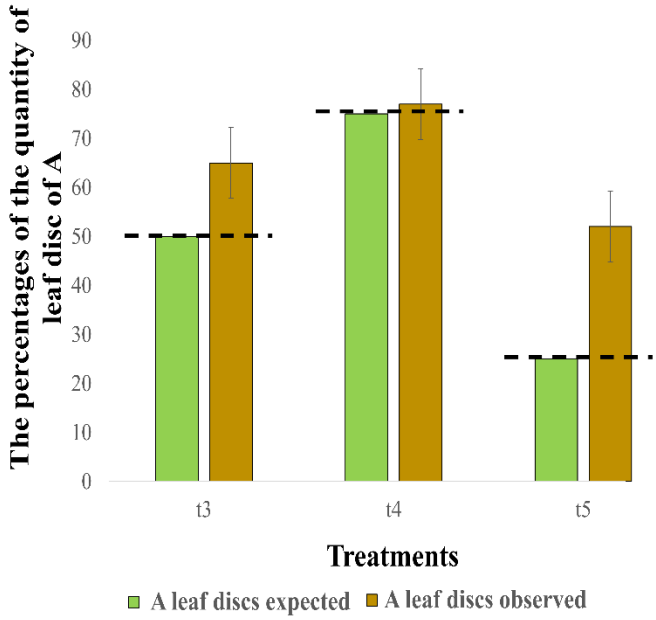


Figure 29. The observed consumption (black bars) of A leaf discs compared with the expected consumption (grey bars). The expected A leaf discs as the initial proportion of A discs at each treatment: t3=50%, t4= 75% and t5= 25%). N=5 (t3, t4) and N=4 (t5).

Discussion

Our results showed that flow intermittency reduces the quality of leaf litter in terms of fungal richness and biomass and lipid content (*Objective 1).* In addition, these changes in food quality influenced the consumption rates (i.e., the leaf litter most consumed were those conditioned under permanent flow) and growth of the shredder (*Objective 2*). Finally, *P. latipennis* fed selectively on higher quality leaves; although its availability (quantity) was lower (*Objective 3*).

Effects of flow regime on leaf litter quality

This study pointed out that flow regime influenced the leaf litter quality by means of changes in fungal colonization. Permanent flow allowed the continuous colonization of leaf litter, resulting in higher fungal richness, biomass and lipid content than leaf litter conditioned under intermittent flow conditions.

According to Suberkropp et al. (Suberkropp *et al.*, 1983), hyphomycete richness affects the palatability of the leaf resources, as a higher leaf litter quality is associated with fungal composition and richness. Several studies reported that shredders preferentially fed on well-conditioned leaf litter (Chung & Suberkropp, 2009; Gonçalves *et al.*, 2016), probably related to the characteristics of the fungi themselves (high nutritional value) (Danger & Chauvet, 2013) and to the chemical modifications of leaf litter by fungi (Bärlocher, 2016). Different fungal species have different degradative capabilities that make leaf litter more palatable. Previous studies (Suberkropp *et al.*, 1983; Danger & Chauvet, 2013) demonstrated that *A. acuminata, C. aquatica, F. curvula, L. aquatica and T. marchalianum* had the

capacity to produce enzymes that degraded polygalacturonic acid, xylan and carboxymethyl cellulose. Our results showed that the abundances of most of these species (except *F. curvula*) were higher on leaf litter conditioned under permanent than intermittent flow condition. Another species, *T. elegans,* has similar enzyme capabilities (Chamier & Dixon, 1982), and this species was more abundant in the permanent stream. Furthermore, *H. lugdunensis* has been reported as a fungal colonizer preferred by shredders (Graça *et al.*, 1993), and this specie appears in a higher abundance in leaf litter colonized under continuous flow. All of these data reinforce the idea that permanent flow conditions promoted a higher fungal richness on leaf litter, and therefore, a higher litter quality than under intermittent flow conditions. To further explore this idea, we suggest that additional studies should be conducted using molecular analysis to evaluate the roles of other low-abundance fungal species that might be relevant in terms of leaf palatability. Such studies will provide knowledge on specific fungal traits and enzymatic activities.

In addition to fungal richness, our results also pointed out that higher leaf-associated fungal biomass occurred under permanent than intermittent flow conditions, which is consistent with previous studies (Graça, 2001; Pascoal *et al.*, 2010; Geraldes *et al.*, 2012). While flow disruption constrained and retarded fungal growth and colonization, permanent flow stimulated the sporulation process and supplied a continuous source of fungal spores to leaf litter (Bärlocher, 2016). A higher fungal biomass is related to a higher litter quality and palatability, attributable to an enrichment of N due to the uptake and immobilization of this element from the water column by fungal communities (Menéndez *et al.*, 2011).

The lower fungal biomass found under intermittent flow conditions also influenced the lipid content, as demonstrated in other studies (e.g., Geraldes *et al.*, 2012; Mas-Martí *et al.*, 2015; Sanpera-Calbet *et al.*, 2017b). Flow intermittency determines a reduction in the total and essential fatty acids in leaf litter (Mas-Martí *et al.*, 2017; Sanpera-Calbet *et al.*, 2017b), which influences its quality. Müller-Navarra et al. (Müller-Navarra *et al.*, 2000) found that the contents of lipids, such as fatty acids, including polyunsaturated fatty acids, is essential and can limit consumer growth, reproduction, neural development and trophic transfer efficiency. In accordance, the higher total lipid concentration found in leaf litter colonized in the permanent stream led to a better quality (Torres-Ruíz *et al.*, 2007; Torres-Ruíz & Wehr, 2010) for consumers.

Finally, molar C:N ratios are considered an important indicator of the nutritional value of food resources due to the positive correlation between nitrogen content and shredder preferences (Leberfinger & Bohman, 2010). Unfortunately, in our results, we did not find significant differences in the C:N ratios for leaf litters conditioned in the two streams.

Effects of the leaf quality and quantity on consumer consumption and growth

Several studies have shown the importance of nutritional quality of the leaf litter resource for consumer feeding preferences and growth. Gonçalves et al. (Gonçalves *et al.*, 2014, 2016) highlighted the importance of fungal composition and richness on shredder feeding rates, and Arsuffi & Suberkropp (Arsuffi & Suberkropp, 1986) showed the importance of lipids and proteins for stimulating shredder consumption, as shredders cannot

synthesize these components and must therefore acquire them from their diet (Cargill *et al.*, 1985b,a).

The results of our experiment showed that despite the differences in leaf quality, the total consumption and RCR between the leaf litters conditioned in both streams were not significantly different. The similar consumption rates observed in our study between the t1 and t2 treatments (100% leaf discs from A and B, respectively) could be related to the fungal composition of the leaf litter in both streams, among other factors. As indicated previously, two fungal species reported as being highly palatable to shredders (*F. curvula and H. lugdunensis)* were abundant in the leaf litters conditioned in both streams (Arsuffi & Suberkropp, 1986; Bärlocher & Kendrick, 1975; Graça, 2001; Graça *et al.*, 1993). However, when we simultaneously offered leaf discs from both streams (treatments t3, t4 and t5), shredders consumed less when A leaf discs were in a lower proportion (t3, t5; 50% and 25%, respectively) in relation with t1 and t4 (100% and 75%, respectively). There was a preferential selection of A leaf discs in all mixture treatments, as demonstrated by the feeding preference results **(Figure 28)**. The higher fungal biomass and lipid content of the A leaf discs together with their fungal composition stimulate the shredder selection of these leaves in mixture treatments.

Fungal biomass accrual on leaves tends to increase the leaf N content, and the enzymatic maceration of leaves by the fungal community results in smaller and less refractory plant polymers, both processes making leaf resources more palatable to shredders (Rong *et al.*, 1995; Chauvet *et al.*, 2016). Nevertheless, other studies show that a high fungal biomass does not necessarily imply a higher palatability of leaves, suggesting that shredder feeding depends on other characteristics, such as the leaf toughness,

nutrient content, presence of mycotoxins and adaptation of shredders to those chemicals (Bärlocher, 1985; Graça, 2001).

The similar consumption rates between A and B leaves did not translate to similar growth rates. The RGR and IGR were lower when only B leaf discs were offered. This result suggests that the consumption rate of B leaf discs was not sufficient to achieve similar growth. Consumers have two ways to compensate for the limitations of a poor resource quality, increasing consumption (feeding compensation; Flores *et al.*, 2014) or increasing assimilation rates, for example, by enhancing the retention time in their guts (Auer *et al.*, 2015). In general, the treatments with leaf mixtures also showed significantly lower growth rates regarding t1 with the exception of t5 for RGR. Our shredder mainly selected A leaf discs in mixture treatments, but the lower availability and/or the presence of less palatable leaf discs from stream B also limited its growth rate.

Food quality affects energy allocation (lipid storage). According to Flores et al. (Flores *et al.*, 2014), the larvae fed poor-quality resources allocated a higher proportion of lipids to their body conditions than to growth. Larvae fed leaves of the poorest quality (from stream B) tended to allocate more lipids than larvae fed leaves of the richest quality (from stream A). Nevertheless, we did not find significant differences.

Finally, our results showed that the leaf quality affected the basal metabolism of the larvae. The basal metabolic rate determines the energetic cost of living, and after meeting the baseline energy requirements, shredders tend to allocate excess energy to other functions, such as growth and reproduction. The larvae fed leaf discs from stream B showed the highest oxygen consumption rate. This higher metabolism leads to lower energy being invested in growth, as shown in our results (Auer *et al.*, 2015).

Consumers tend to maximize their feeding preferentially on food resources that are energetically most profitable (Kaspari *et al.*, 2012) . They meet their elemental composition requirements to optimize their growth and reproduction, feeding preferentially on high-quality resources (Sterner & Elser, 2002; Woodward, 2009), and our findings are consistent with these statements. While the response of shredders has been strongly related to resource quality, what happens when high-quality resources are scarce remains in question. Other questions remaining include whether consumers actively search for high-quality resources even though they are the least abundant or whether they prefer to consume without selection and exert a more efficient assimilation to maintain homeostasis. Cruz-Rivera et al. (Cruz-Rivera *et al.*, 2008) suggested that resource selection seems to be related to the mobility of organisms. Our results suggest that when mobility is not a handicap, shredders seemed to actively select the food of better quality based on the quality properties despite its lower abundance, although this can limit their growth. We hope this finding stimulates future research to explore how mobility and resource availability interact in shredders.

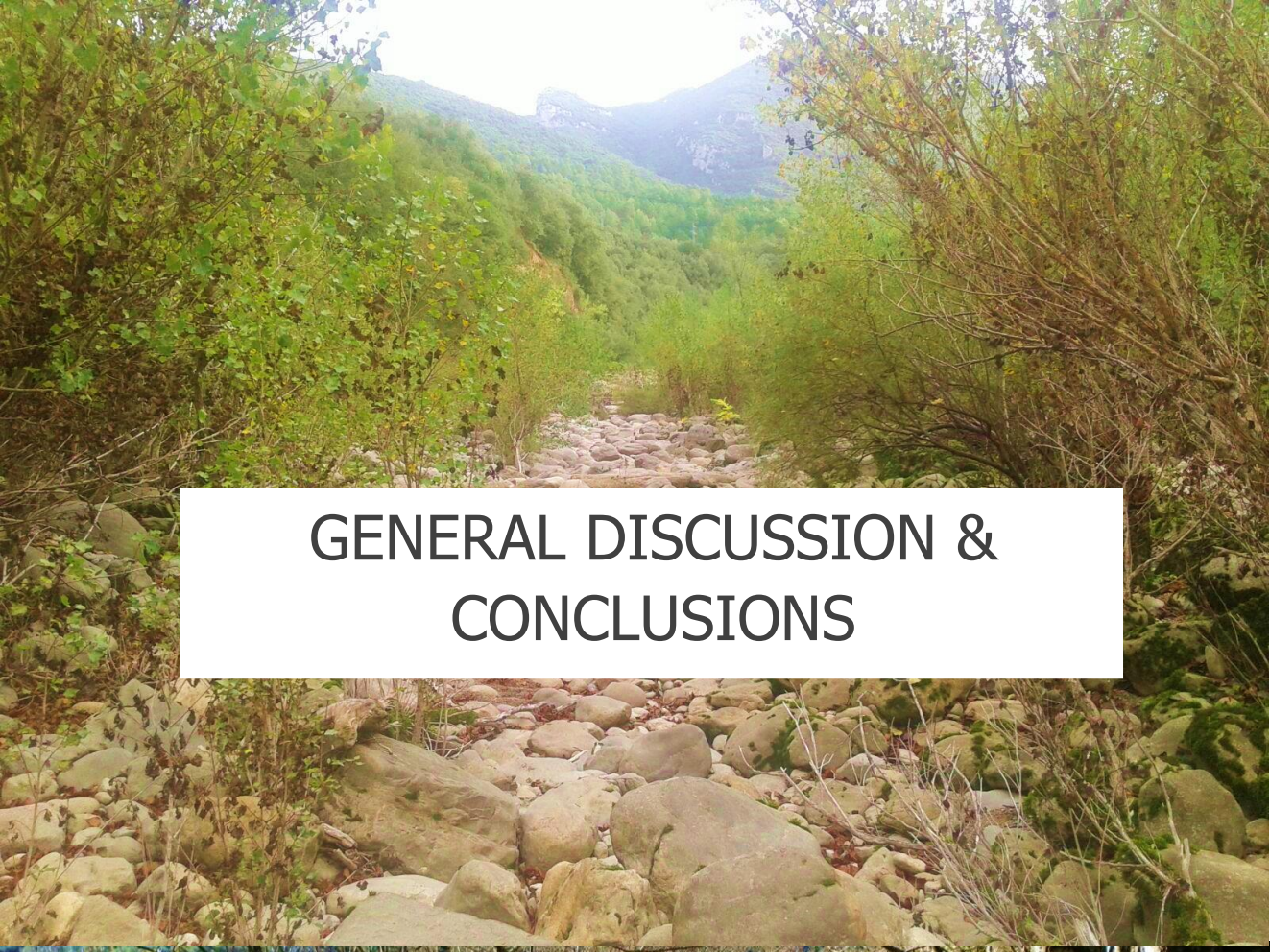
In the context of increasing global water demand and aridification, flow intermittency will become more frequent, leading to drastic changes in food quality and quantity in rivers. Our findings demonstrate that such changes affect the fungal colonization of leaf litter, reducing several litter quality properties and ultimately affecting shredder consumption rates, growth and feeding selections. These responses could therefore potentially threaten the entire fluvial food web. These results provide a better understanding of the effects of changes in flow conditions on ecosystem functioning (leaf litter processing) in rivers and warn of the importance of guaranteeing the natural hydrological dynamics via a better management of water use.

Acknowledgements

We gratefully acknowledge Julio C. López-Doval for his help in the lipid analyses, Veronica Granados for her help in the field, and especially Cayetano Gutierrez-Cánovas for his help with the statistical analyses in R.

Microcosms experiment.

Example of sampling sites.



GENERAL DISCUSSION & CONCLUSIONS

A MÍ NADIE ME CONTO…..QUE ESTO DE VIVIR ES UNA ETERNA DESPEDIDA….

-Jose ft. Pedro Pastor y los locos descalzos-

GENERAL DISCUSSION

It is now recognized that flow regimes are changing worldwide in response to climate change and the increasing water demands of humans (Kummu *et al.*, 2016; Koutroulis *et al.*, 2019). As a result, there is an ongoing expansion in global IRES distribution and their non-flow period continues to increase, both spatially and in duration (Döll & Schmied, 2012). Despite their widespread occurrence, adequate characterization of the flow intermittence complexity is not yet consolidated, which limits advances in IRES management and protection.

This thesis contributes to remedying this shortage of knowledge through *in situ* characterization of stream hydrology over one year and a half before sampling campaigns to capture the different aspects of flow intermittence. That characterization is performed using metrics that describe intermittence regime (total non-flow days and number of non-flow events) and intermittence status (duration of the last non-flow period and time since rewetting). Such detailed characterization of flow intermittence has allowed me to understand changes in microbial decomposition better, using this as a proxy of ecosystem function in both the surface and subsurface zone, as well as changes in aquatic diversity response (invertebrates and aquatic hyphomycetes).

This discussion combines the main results obtained in the different chapters, situating them within a wider context. I also discuss their concordance with my initial hypotheses and their applied implications. Overall, I have shown that flow intermittence reduces the diversity of invertebrates and aquatic hyphomycetes, affecting organic matter decomposition and the energy flow to higher trophic levels. These effects seem to arise because flow

intermittence affects invertebrate trophic groups and both the quality and quantity of leaf litter.

The discussion is divided into four sections. The first section focuses on the characterization of flow intermittence and the importance of metrics in structuring stream diversity and ecosystem functioning. In addition, in this section I discuss how my hydrological metrics can contribute to improving IRES classification and bio-monitoring routines. The second section focuses on the role of the subsurface zone in sustaining organic matter decomposition during non-flow periods. The third section focuses on the responses of invertebrate and aquatic hyphomycete diversity to flow intermittence and their ecological implications. Finally, the last section focuses on future research aimed at addressing unanswered questions.

The importance of measuring *in situ* flow intermittence

Flow is the master variable that governs biotic structure and the functioning of fluvial ecosystems, and it shows great spatial and temporal variability (Lytle & Poff, 2004; Palmer & Ruhi, 2019). Regarding temporal scales, we can find different annual and recent hydrological dynamics that may influence the type of organisms that can thrive in a stream, and the performing of ecosystem processes such as organic matter decomposition, nutrient cycling, primary and secondary production or energy transfer to higher trophic levels (Tonkin *et al.*, 2017; Rolls *et al.*, 2018). On one hand, annual flow intermittence dynamics determine the number of days in which abiotic conditions allow organisms to colonize and develop in a given habitat

(Bruno *et al.*, 2016a). On the other hand, recent intermittence dynamics vary seasonally and determine the moment when habitats can be colonized, and the window for growth and reproduction. Both hydrological scales act as effective filters for aquatic organisms and ecosystem processes (McMullen *et al.*, 2017; Palmer & Ruhi, 2019). For example, daily flow conditions affect ecological processes through changes in abiotic characteristics such as water temperature, oxygen dissolved in the water or nutrient availability (Poff *et al.*, 1997; Granados *et al.*, 2020).

Despite the role of temporal scaling of hydrology on biodiversity and ecosystem functioning, IRES have usually been characterized by taking into account just the annual scale, based on the non-flow period, to define or discriminate classes of intermittency. Furthermore, most studies characterize flow intermittence based on categorical classes, e.g., permanent, intermittent, ephemeral; or the connected vs. disconnected status of rivers and streams (Gallart *et al.*, 2012, 2017); or through mean annual values for non-flowing days, obtained from hydrological models (e.g. Belmar *et al.*, 2019). Such approaches have a limited capacity to link flow intermittence, biota and ecosystem processes, as they do not consider the continuity of intermittence, or they ignore inter-annual variability or the recent history of intermittence events and their duration (Patrick & Yuan, 2017; Palmer & Ruhi, 2019).

Taken together, my findings demonstrate the importance of measuring *in situ* flow intermittence metrics to capture more precisely intermittence aspects such as annual intermittence regime (total non-flow days and number of non-flow events) and recent disturbance conditions (duration of the last non-flow period and time since rewetting) (Chapters 1, 2 and 3).

In Chapter 1, I have showed that even considering just total non-flow days, it is necessary to know the real number of non-flow days exactly, in order to estimate and discriminate the contribution of subsurface and surface zones to microbial decomposition better. Regarding communities, the importance and the effect of these metrics seem to be related to the mobility of the organisms and their life cycles duration. For example, invertebrate diversity metrics, either taxonomic or functional, have been explained by a combination of annual and recent aspects of flow intermittence (Chapter 2); while for aquatic hyphomycetes, they are mainly explained by annual aspects (Chapter 3). These findings suggest that the longer life cycles of invertebrates could make it more difficult for them to thrive over shorter rewetting periods, as opposed to hyphomycetes (Read *et al.*, 1992), which show different responses to recent intermittence conditions. Microbes usually have quicker response capacities as a result of their short life cycles and their high capacity to restore their activity (Foulquier *et al.*, 2015; Gionchetta *et al.*, 2019), i.e., spore production rapidly resume in just a few hours on rewetting, leading to a recovery of the community (Chapter 3).

Overall, the two intermittence components seem to play different but complementary roles in structuring stream biodiversity and, consequently, in sustaining ecosystem functioning. Therefore, studies that consider categorical or less accurate hydrological descriptors would have a limited capacity to predict ecosystem trajectories in response to global change.

Finally, according to my expectations, intermittence descriptors generally outweighed non-hydrological descriptors when it came to explaining biodiversity aspects. This support the use of the former in IRES classification and bio-monitoring routines.

The importance of the subsurface zone to maintain ecosystem function during non-flow periods

In a wider context, dry streambeds and subsurface zones are important components of biogeochemical cycles, and particularly for carbon (C) processing. Recent studies have demonstrated that dry streambeds release considerable amounts of carbon dioxide (CO_2) into the atmosphere. These emissions result in even higher emission rates than those produced by flowing streams, mainly due to the microbial activity that remains during the non-flow period (Gómez-Gener *et al.*, 2016; Arce *et al.*, 2019; Gionchetta *et al.*, 2019; Marcé *et al.*, 2019; von Schiller *et al.*, 2019). Furthermore, in dry streambeds, particulate organic matter is accumulated during drying and could be buried within the subsurface zone during storms (Scott & Zhang, 2012). As a result, the subsurface zone may be the most important organic matter storage compartment in at stream (Storey *et al.*, 1999; Cornut *et al.*, 2012). Furthermore, flow intermittence determines a patchy distribution of habitats during flow cessation, which act as a natural filter for aquatic organisms (Lake, 2000, 2003; Larned *et al.*, 2010) among those micro-habitats, depending on their degree of adaptation to dryness. When water flow ceases, leaf packs and especially sediments represent the last wet habitats available to organisms playing an important role as a refuge for aquatic organisms (Stubbington, 2012; Chauvet *et al.*, 2016; Arce *et al.*, 2019; Gionchetta *et al.*, 2020).

Previous studies have focused either on surface or on subsurface zones (Burrows *et al.*, 2017; Datry *et al.*, 2018b), rather than simultaneously considering and comparing the two. My results demonstrate the importance

of simultaneously studying both zones, in order to further our understanding of how microbial decomposition may respond to increasing intermittence (Chapter 1). Organic matter processing in both zones decreased with increasing non-flow duration; but streams undergoing more than 75 non-flow days maintained roughly the same decomposition rates in the subsurface zone as that which occurs in the surface zone of streams with fewer than 75 non-flow days. Hence, the subsurface zone remains biologically active during the non-flow period, maintaining the process of organic matter decomposition. One explanation for this could be that fungal communities are hosted in the subsurface zone. Indeed, I have showed that fungal biomass in the subsurface zone increases with the intermittency. The other explanation would be that during non-flow periods, the subsurface has more constant physicochemical conditions than the surface zone, and it could remain saturated with water for longer periods (Martínez *et al.*, 2015). These stable conditions are important to maintain the activity of microorganism (Arce *et al.*, 2019). In addition, my results suggest that the magnitude of microbial decomposition and fungal biomass in the subsurface zone depend on local environmental factors such as sediment grain size, water content and sediment C:N ratios.

Effects of flow intermittence on IRES diversity and their implications for ecosystem functioning

Flow regimes shape the taxonomic and functional diversity of aquatic organisms (Poff *et al.*, 1997; Lytle & Poff, 2004; Tonkin *et al.*, 2017, 2018).

Over evolutionary time, organisms developed traits that have enabled them to survive natural flow disturbances, such as floods or droughts (Rolls *et al.*, 2018; Palmer & Ruhi, 2019). Examples of adaptations conferring resistance and resilience to intermittence are short life spans that suit short windows of flowing water, and terrestrial reproduction or aerial respiration to gain independence from aquatic conditions. As human activities are altering natural flow disturbances, it is necessary to understand how aquatic communities respond to flow intermittence and its implications for ecosystem functioning (Lytle & Poff, 2004; Tonkin *et al.*, 2017), in order to predict and prevent effects in future scenarios. Despite the growing scientific literature that addresses the effect of flow intermittence on diversity, especially on invertebrate communities (see references in Chapter 2), it is unclear how different temporal characteristics of the hydrological disturbance influence aquatic organisms. This limits our capacity to predict ecosystem trajectories in response to global change (Chapters 2 and 3). In part, this inconsistency emerges from the incomplete characterization of flow intermittence components (see the section above) and because aquatic communities have only partially been described, with more focus on structure (taxonomy) than function, and more on invertebrates than other aquatic communities (e.g., fungal communities) (Chapters 2 and 3).

This thesis contributes to remedying this gap in our knowledge by using a multi-model inference approach to test the predictive capacity of different flow intermittence components, alone and in combination, together with non-hydrological environmental variability. I have found that both annual and recent flow intermittence disturbances shape different aspects of aquatic diversity, which determine how stream communities assemble and support ecosystem functioning.

Invertebrate communities

As illustrated in Chapter 2, longer periods or a higher frequency of non-flow days and shorter rewetting periods reduces the diversity and abundance of aquatic invertebrate communities and trophic groups. In contrast, the non-hydrological variables were less influential than the hydrological ones in shaping biological communities, which agrees with my initial hypothesis. These results suggest that IRES communities are more tightly linked to hydrological disturbances than geomorphological, land-use or water chemistry variables, even though this latter group of predictors is commonly used to predict and typify biological communities. Among trophic groups, predators, grazers, shredders and filterers showed more pronounced responses to intermittency (Chapter 2). As I show in **Figure 5** (general introduction), the reduction in these trophic groups will reduce the efficiency of organic matter decomposition and possibly other ecosystem functions, potentially threatening the entire fluvial food web.

Non-flow periods can limit the occurrence of certain taxa that are unable to complete their life cycle or to cope with chemical stress due to desiccation (Stubbington & Datry, 2013; McMullen *et al.*, 2017; Granados *et al.*, 2020). The time since rewetting can affect taxa recovering from dormancy or resistance stages, and also the recovery of habitat and food resources, as I have shown for trophic diversity (Chapter 2, Leigh & Datry, 2017). All of these results suggest strong habitat filtering in IRES that sorts species in relation to their tolerance to desiccation stress and other abiotic stressors (Odum, 1985; Gutiérrez-Cánovas *et al.*, 2013, 2015; Soria *et al.*, 2020). For example, in the case of invertebrates, longer non-flow periods tend to select a reduced set of fast living, multivoltine organisms (Belmar et al., 2019; Díaz, Alonso, & Gutiérrez, 2008), which are prone to develop and maintain large

stocks of individuals through various reproductive events within a year (Chapter 2).

Aquatic hyphomycetes

As shown in Chapters 3 and 4, I found that longer non-flow periods lead to reduced aquatic hyphomycete richness and composition. Such changes affect the fungal colonization of leaf litter, reducing several litter quality properties and ultimately affecting shredder consumption rates, food selection and growth.

In contrast to the case of invertebrates, even when considering background environmental variability and multiple intermittence metrics, the total number of non-flow days emerges as the variable that explains most aquatic hyphomycete alpha and beta diversity (Chapter 3). The relative lack of importance of recent aquatic status could be due to the capacity of microbes to respond quickly to short-term disturbances as a result of their short life cycles and highly dynamic populations (Bärlocher, 2009; Grimmett *et al.*, 2013). For instance, even some short-lived storms can stimulate and restore their activity rapidly (Bruder *et al.*, 2011). These changes in alpha and beta diversity affect the palatability of leaf resources, as greater leaf litter quality is associated with fungal composition and richness, since different fungal species have different degradation capabilities that make leaf litter more palatable (Chapter 4). Thus, flow disruption reduces leaf quality, through a limited lipid content, which arises as a result of retarded fungal growth and colonization. It is known that this reduction in lipid content is negative for invertebrate consumers, as the lower content affects consumer growth, reproduction, neural development and trophic transfer efficiency. The higher fungal biomass and lipid content of leaves together with their fungal

composition stimulate shredder selection of these resources and their consumption, irrespectively of quantity (Chapter 4). Therefore, changes in fungal diversity affect organic matter quality and processing through reduced fungal colonization, which reduces shredder activity, feeding selection and growth, and has an impact not only on overall decomposition rates, but potentially on the entire fluvial food web.

In addition, in line with my prediction, I have demonstrated that compositional changes arise from species replacement, suggesting that aquatic hyphomycetes have a strong affinity for different intermittence regimes. The fact that compositional changes are explained by species replacement, and not by nestedness, suggests that resistance mechanisms rather than resilience-related adaptations could explain how hyphomycetes evolved to cope with flow intermittence. The high specificity of aquatic hyphomycetes for their organic substrates and their life cycle under aquatic conditions could reinforce the idea that resistance and specialization mechanisms explain compositional changes. Thus, exploring beta diversity patterns has been a useful approach to disentangle the roles of community resistance and resilience mechanisms in IRES, for a group for which functional traits are largely unknown.

Future research

The rapid acceleration of IRES research over recent years represents an opportunity for great advances in our understanding and the management of these systems, through international collaborations between aquatic and terrestrial ecologists and hydrologists. Nevertheless, there are still many gaps in our knowledge and understanding of IRES.

1. Improving, harmonizing and fostering hydrological characterization of IRES

One of the major challenges we face when we aim to understand how flow intermittence affects biodiversity and ecosystem functions is to provide adequate characterization of the different flow intermittence components and to foster harmonized approaches, hydrological metrics and river classifications. Thus, future research should address these challenges by focusing on the development of technologies for gathering real-time hydrological data that track the presence or absence of water. The use of low-cost sensors that detect the presence of water (i.e., through electrical conductivity, water temperature or water level) or field cameras (similar to those used to monitor fauna in terrestrial ecology), regular site visits (or at least the use of remote sensing data from the sites) and citizen science (e.g., CrowdWater, https://crowdwater.ch/en/crowdwaterapp-en/) will contribute to a better hydrological characterization of IRES. In addition, future studies should focus on the associated flow intermittence and hydrological connectivity across the three spatial dimensions, taking into account the subsurface zone which ensures fluvial ecosystem functions.

2. Enhanced functional and mechanistic characterization of IRES communities

Another future challenge will be to improve the functional characterization of aquatic communities which have only partially been described. This gap of knowledge currently hinders our capacity to infer their mechanistic relationships with flow intermittence and ecosystem functions. Thus, to understand the links between flow intermittence, biodiversity and ecosystem functioning better, I suggest the use of the response-effect trait framework, which distinguishes morphological, behavioral and life-history traits to cope

with intermittence filtering (response traits) and others that support ecosystem function (effect traits).

3. Exploring trophic cascading effects of flow intermittence

Flow intermittence reduces the quality of food resources, through reductions in fungal richness, biomass and the lipid content of organic matter resources. However, its impact on wider food webs is not yet fully understood. To further explore this idea, I suggest that additional studies be conducted using molecular analysis to evaluate the trophic roles of rare fungal species that might be relevant in terms of palatability. Such studies would provide knowledge on specific fungal traits and enzymatic activities, which in addition could help to develop a data base of fungal response and effect traits. That would allow us to understand their mechanistic relationships with flow intermittence and ecosystem functioning better. Furthermore, I hope that my findings stimulate future research to explore invertebrate consumer' mobility and resource availability and thereby to understand the impact of intermittence-related alterations to food quality better.

4. Exploring the role of the micro-habitat at the reach scale

A shift to a drier climate may lead to an increase in the frequency and duration of flow interruption in many areas, which could compromise the functional stability of ecosystems. Thus, considering the key role of biodiversity in ecosystem processes, future studies should identify biodiversity targets and implementing measures to protect refuges in stream reaches as a source for recolonization to sustain ecosystem functioning. I hope that my framework based on different intermittence measures and a

wide characterization of biological communities provides managers and researchers with tools to predict better how stream communities respond to flow intermittency in a more arid world.

CONCLUSIONS

This thesis contributes to a better understanding of the effects of flow intermittence on biodiversity and ecosystem functioning. The results highlight the necessity of taking into account different flow intermittence components that should be measured *in situ* for a better characterization of IRES. I now summarize the main thesis conclusions for each chapter.

CHAPTER 1: Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period.

1. **Subsurface processes** make an **important contribution** to **sustaining microbial decomposition during the non-flow periods** of IRES.
2. **Organic matter decomposition** in **surface and subsurface zones decreases** with **increased intermittency, but** the **subsurface zone** could **maintain organic matter decomposition** as the **period without flow lengthens**; for instance, streams that undergo more than 75 days of no flow could maintain approximately the same decomposition rates as the surface zones in streams with fewer than 75 days of no flow. Thus,

simultaneously studying both zones is crucial to furthering our **understanding of how microbial decomposition may respond to the expected increase in the duration and severity of flow intermittence** as a result of climate change.

3. **An increase** in the number of **non-flow days** is related to lower organic matter decomposition in the **surface zone** due to the **decrease** in **fungal biomass;** whereas in the **subsurface zone, fungal biomass increases as the number of non-flow days increases**.
4. Although the total number of **non-flow days** is the **main driver** of both **microbial decomposition and fungal biomass, the magnitude of these parameters depends on the local environmental factors of rivers and streams**, such as soluble reactive phosphorus in the surface zone, and sediment grain size, water content and sediment C:N ratio in the subsurface zone.
5. **Dry streambeds** and **subsurface zones must be considered to ensure fluvial ecosystem functioning.**

CHAPTER 2: Annual and recent flow-intermittence disturbances explain the high variability of stream invertebrate diversity.

1. **Annual and recent flow intermittence disturbances shape** different aspects of stream **invertebrate communities**, which determine how stream communities assemble and support ecosystem functioning.

2. **Longer periods or a higher frequency of non-flow days** and **shorter rewetting** periods can **reduce the diversity and abundance of invertebrate** communities **and trophic groups**, with potential negative consequences for ecosystem functioning.
3. **Richness and diversity-type metrics**, either taxonomic or functional, are explained **by a combination of annual** (frequency of non-flow periods and total number of non-flow days) **and recent** (rewetting duration) **flow intermittence disturbances,** whereas **abundance-based measures** are primarily determined by changes in **total non-flow days.** These patterns reflect the fact that the amount of organisms in a community or trophic group depends on a longer temporal scale than taxon or trait richness metrics, where rewetting events could have enabled a more diverse representation of organisms.
4. **Trophic strategies have differential responses to intermittency,** probably due to direct intolerance of **harsh environmental conditions,** mediated by response-traits; or because of trophic cascading effects due to alterations in **basal resources.**
5. **Intermittence descriptors generally outweigh environmental descriptors** in explaining biodiversity aspects. This support the use of the former in IRES classification and bio-monitoring routines. My framework, based on intermittence measures and different characteristics of biological communities, could help improve predictions of how stream communities will respond to flow intermittency in a more arid world.

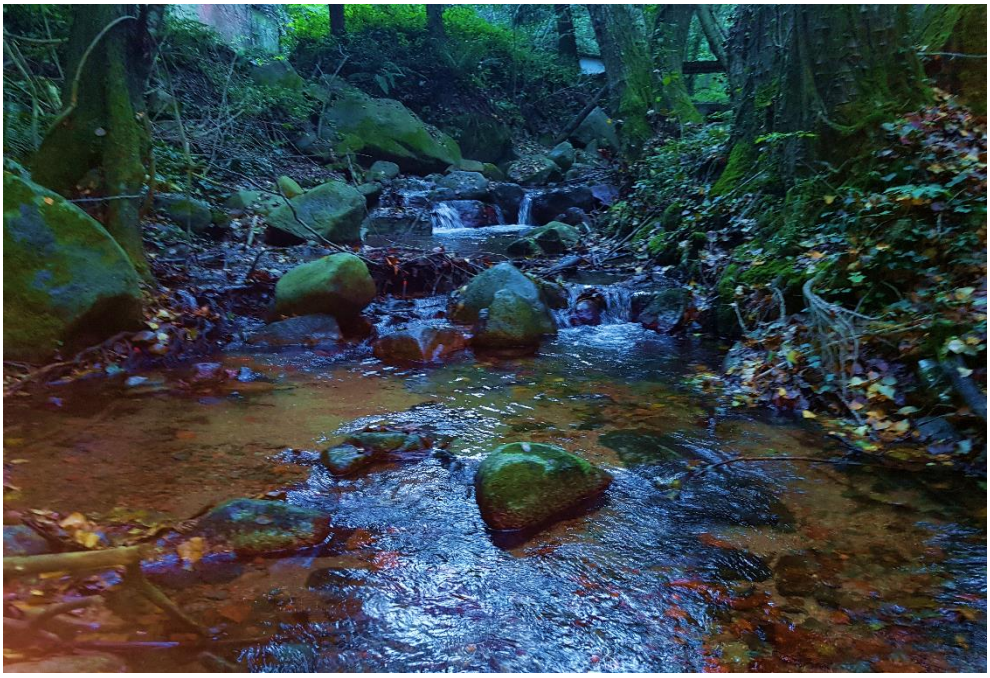
CHAPTER 3: Flow intermittence drives aquatic hyphomycete turnover and richness reduction which affects organic matter decomposition.

1. **Annual flow intermittence** disturbances **shape aquatic hyphomycete** diversity, both **alpha and beta diversity**.
2. **Longer non-flow periods** lead to **reduced** aquatic hyphomycete **richness**.
3. **Aquatic hyphomycete richness mediates or attenuates** the **effect of flow intermittence on organic matter decomposition**.
4. **Beta diversity patterns** are a **useful approach** to disentangle the **roles of community resistance and resilience mechanisms in IRES** for a group whose **functional traits are** largely **unknown**.
5. Compositional changes arise from **species replacement**, revealing **resistance adaptation mechanisms are more prevalent.**

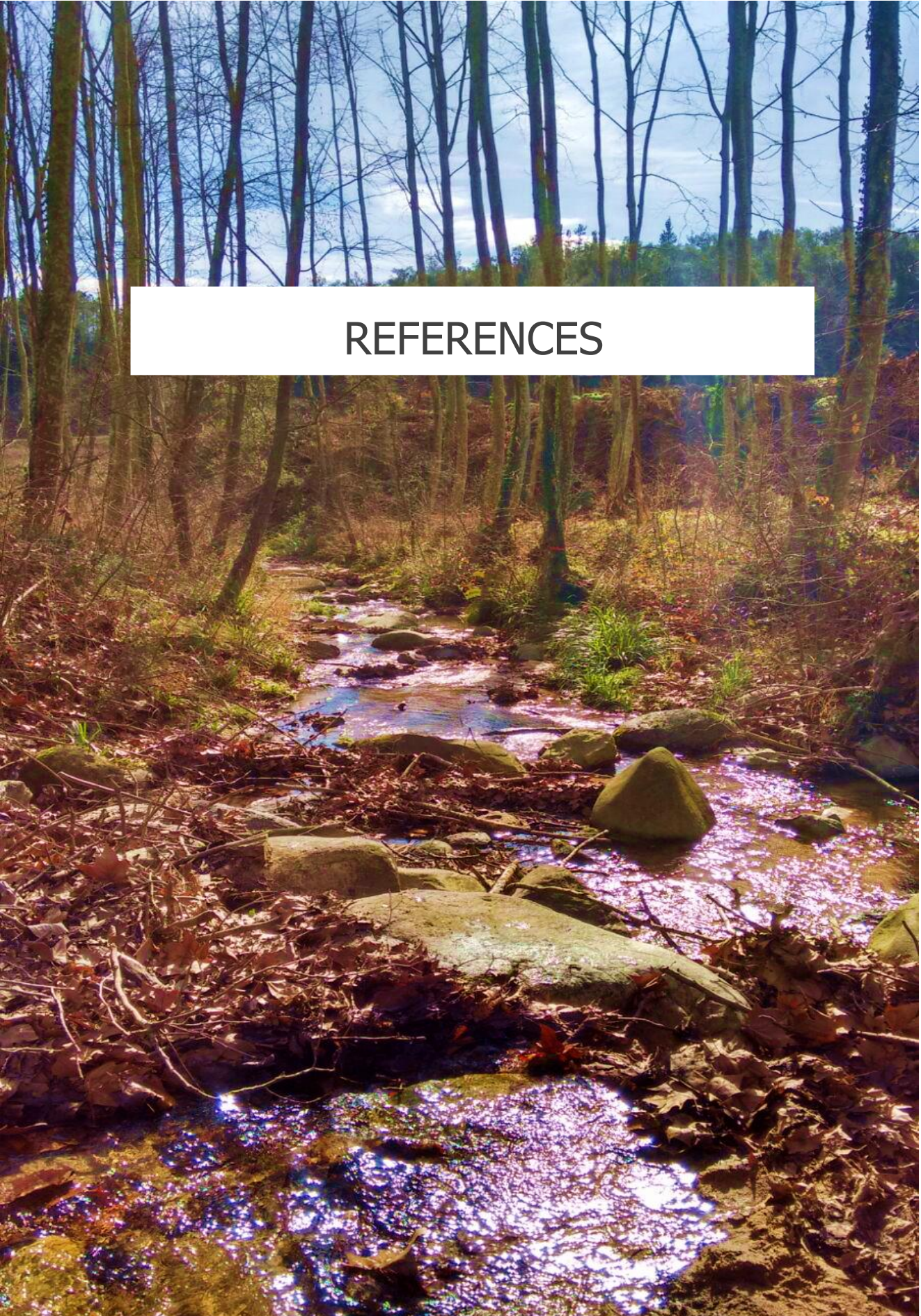
CHAPTER 4: Quality and quantity of leaf litter: both are important for feeding preferences and growth of an aquatic shredder.

1. **Flow intermittence reduces fungal richness, fungal biomass and lipid content** affecting the **palatability of the resource**. This is probably related to the characteristics of the fungi themselves (high nutritional value) and to the chemical modifications of the resource by fungi (increasing nitrogen and lipids content).

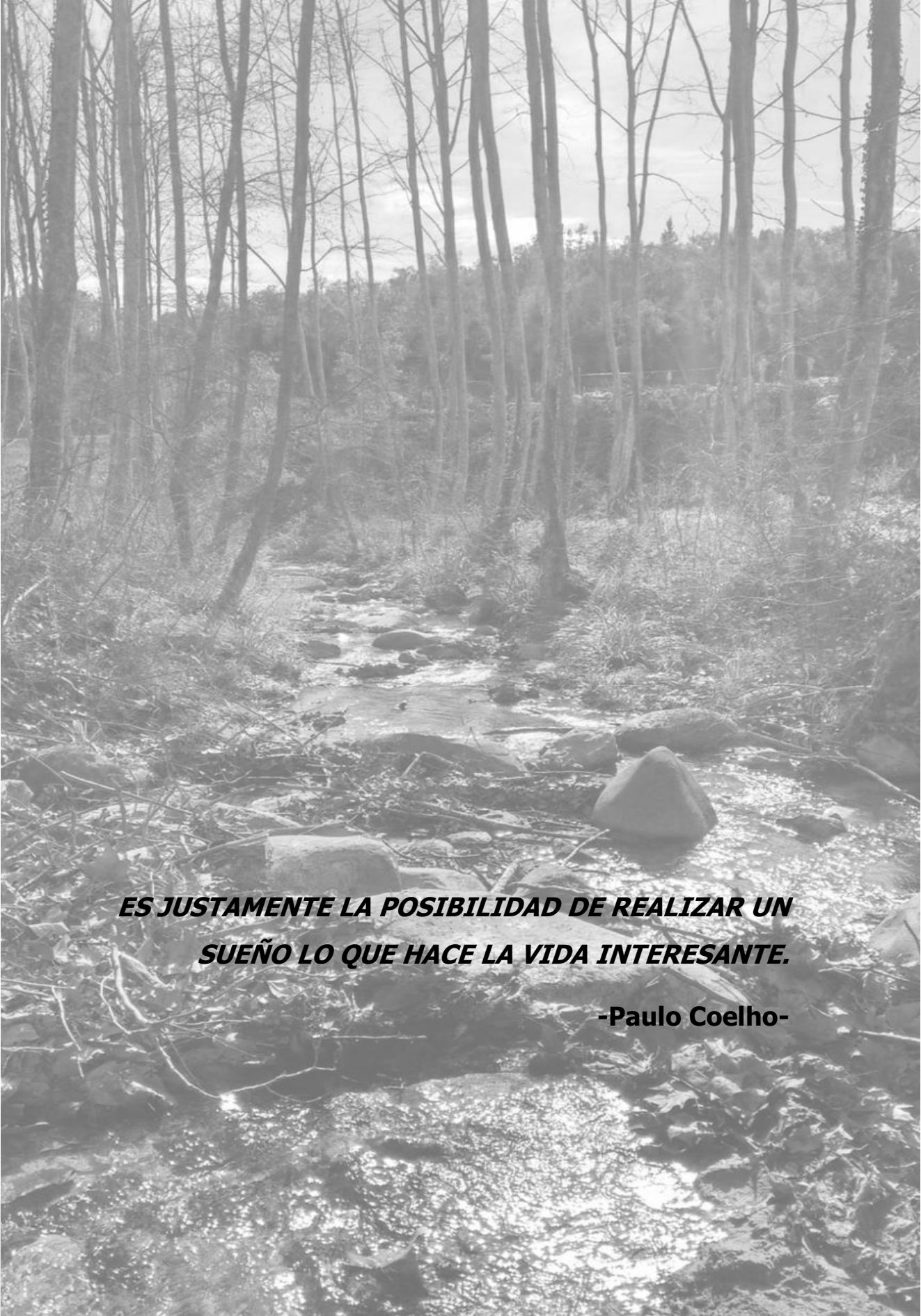
2. **Changes** in **resource quality influence** the **consumption rates and growth** of *Potamophylax latipennis*. Higher fungal biomass and lipid content together with their fungal composition stimulate shredder selection of resources.
3. **Lower leaf quality increases the metabolism of the shredders** leading to lower energy invested in growth.
4. **Consumers** tend to maximize their **preferential feeding** on food resources that are energetically most profitable, i.e., **high-quality resources.** *Potamophylax latipennis* **feeds selectively** on **higher quality leaves**, **although their availability (quantity) was lower.**



Example of sampling sites.



REFERENCES



ES JUSTAMENTE LA POSIBILIDAD DE REALIZAR UN SUEÑO LO QUE HACE LA VIDA INTERESANTE.

-Paulo Coelho-

Abril, M., Muñoz, I. & Menéndez, M. (2016) Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. *Science of the Total Environment*, **553**, 330–339.

Akaike (1973) Information theory and an extension of themaximum likelihood principle. In: Petrov, B.N. (Ed.), Proceedings of the Second International Symposium on Information Theory. *Akademiai Kiado, Budapest, pp. 267–281.*, 267–281.

Anna, A., Yorgos, C., Konstantinos, P. & Maria, L. (2009) Do intermittent and ephemeral Mediterranean rivers belong to the same river type? *Aquatic Ecology*, **43**, 465–476.

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 Reviews*, **188**, 441–453.

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(12): e0**, 1–17.

Arias-Real, R., Muñoz, I., Gutiérrez-Cánovas, C., Granados, V., Lopez-Laseras, P. & Menéndez, M. (2020) Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period. *Science of the Total Environment*, **703**, 135485.

Arroita, M., Aristi, I., Flores, L., Larrañaga, A., Díez, J., Mora, J., Romaní,

A.M. & Elosegi, A. (2012) The use of wooden sticks to assess stream ecosystem functioning: Comparison with leaf breakdown rates. *Science of the Total Environment*, **440**, 115–122.

Arscott, D.B., Larned, S., Scarsbrook, M.R. & Lambert, P. (2010) Aquatic invertebrate community structure along an intermittence gradient: Selwyn River, New Zealand. *Journal of the North American Benthological Society*, **29**, 530–545.

Arsuffi, T.L. & Suberkropp, K. (1986) Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. *Journal of the North American Benthological Society*, **5**, 297–305.

Arsuffi, T.L. & Suberkropp, K. (1984) Leaf processing capabilities of aquatic hyphomycetes: interspecific differences and influence on shredder feeding preferences. *Oikos*, **42**, 144–154.

Artigas, J., Romaní, A.M., Graudes, A., Muñoz, I. & Sabater, S. (2009) Organic matter availability structures microbial biomass and activity in a Mediterranean stream. *Freshwater Biology*, **54**, 2025–2036.

Artigas, J., Romanı, A.M. & Sabater, S. (2008) Effect of nutrients on the sporulation and diversity of aquatic hyphomycetes on submerged substrata in a Mediterranean stream. *Aquatic botany*, **88**, 32–38.

Aspin, T.W.H., Khamis, K., Matthews, T.J., Milner, A.M., O'Callaghan, M.J., Trimmer, M., Woodward, G. & Ledger, M.E. (2019) Extreme drought pushes stream invertebrate communities over functional thresholds. *Global Change Biology*, **25**, 230–244.

Aspin, T.W.H., Matthews, T.J., Khamis, K., Milner, A.M., Wang, Z., O'Callaghan, M.J. & Ledger, M.E. (2018) Drought intensification drives

turnover of structure and function in stream invertebrate communities. *Ecography*, **41**, 1992–2004.

Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J. & Metcalfe, N.B. (2015) The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Functional Ecology*, **29**, 479–486.

Azevedo, M.M. & Cássio, F. (2010) Effects of metals on growth and sporulation of aquatic fungi. *Drug and Chemical Toxicology*, **33**, 269–278.

Baert, J.M., Janssen, C.R., Sabbe, K. & De Laender, F. (2016) Per capita interactions and stress tolerance drive stress-induced changes in biodiversity effects on ecosystem functions. *Nature Communications*, **7**, 1–8.

Bärlocher, F. (2016) Aquatic hyphomycetes in a changing environment. *Fungal Ecology*, **19**, 14–27.

Bärlocher, F. (2009) Reproduction and dispersal in aquatic hyphomycetes. *Mycoscience*, **50**, 3–8.

Bärlocher, F. (1985) The role of fungi in the nutrition of stream invertebrates. *Botanical Journal of the Linnean Society*, **91**, 83–94.

Bärlocher, F., Kebede, Y.K., Gonçalves, A.L. & Canhoto, C. (2013) Incubation Temperature and Substrate Quality Modulate Sporulation by Aquatic Hyphomycetes. *Microbial Ecology*, **66**, 30–39.

Bärlocher, F. & Kendrick, B. (1975) Assimilation efficiency of Gammarus pseudolimnaeus (Amphipoda) feeding on fungal mycelium or autumn-shed leaves. *Oikos*, **26**, 55–59.

Bärlocher, F. & Sridhar, K.R. (2014) *Association of animals and fungi in leaf decomposition*, (ed. by E.B.G. Jones), K.D. Hyde), and K.L. Pang) Freshwater Fungi and Fungal-Like Organisms, De Gruyter, Berlin.

Barnard, R.L., Osborne, C.A. & Firestone, M.K. (2015) Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. *The ISME Journal*, **9**, 946–957.

Baschien, C., Marvanová, L. & Szewzyk, U. (2006) Phylogeny of selected aquatic hyphomycetes based on morphological and molecular data. *Nova Hedwigia*, **83**, 311–352.

Baselga, A. (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134–143.

Baselga, A. & Orme, C.D.L. (2012) betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, **3**, 808–812.

Belliveau, M.J.-R. & Bärlocher, F. (2005) Molecular evidence confirms multiple origins of aquatic hyphomycetes. *Mycological Research*, **109**, 1407–1417.

Belmar, O., Bruno, D., Guareschi, S., Mellado-Díaz, A., Millán, A. & Velasco, J. (2019) Functional responses of aquatic macroinvertebrates to flow regulation are shaped by natural flow intermittence in Mediterranean streams. *Freshwater Biology*, **64**, 1064–1077.

Belmar, O., Velasco, J. & Martinez-Capel, F. (2011) Hydrological classification of natural flow regimes to support environmental flow assessments in intensively regulated Mediterranean rivers, Segura River Basin (Spain). *Environmental Management*, **47**, 992–1004.

Besemer, K. (2015) Biodiversity, community structure and function of

biofilms in stream ecosystems. *Research in microbiology*, **166**, 774–781.

Blazewicz, S.T.J.B., Chwartz, E.G.S. & 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.

Boersma, K.S., Bogan, M.T., Henrichs, B.A. & Lytle, D.A. (2014) Invertebrate assemblages of pools in arid-land streams have high functional redundancy and are resistant to severe drying. *Freshwater Biology*, **59**, 491–501.

Bogan, M.T., Boersma, K.S. & Lytle, D.A. (2013) Flow intermittency alters longitudinal patterns of invertebrate diversity and assemblage composition in an arid-land stream network. *Freshwater Biology*, **58**, 1016–1028.

Bogan, M.T. & Lytle, D.A. (2007) Seasonal flow variation allows "time-sharing" by disparate aquatic insect communities in montane desert streams. *Freshwater Biology*, **52**, 290–304.

Bonada, N. & Dolédec, S. (2011) Do mediterranean genera not included in Tachet et al. 2002 have mediterranean trait characteristics? *Limnetica*, **1**, 129–142.

Booker, D.J., Snelder, T.H., Greenwood, M.J. & Crow, S.K. (2015) Relationships between invertebrate communities and both hydrological regime and other environmental factors across New Zealand's rivers. *Ecohydrology*, **8**, 13–32.

Boulton, A.J. (2003) Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages. *Freshwater Biology*, **48**, 1173–

1185.

Boulton, A.J., Datry, T., Kasahara, T., Mutz, M. & Stanford, J.A. (2010a) Ecology and management of the hyporheic zone : stream – groundwater interactions of running waters and their floodplains. *Journal of the North American Benthological Society*, **29**, 26–40.

Boulton, A.J., Datry, T., Kasahara, T., Mutz, M. & Stanford, J.A. (2010b) Ecology and management of the hyporheic zone: stream–groundwater interactions of running waters and their floodplains. *Journal of the North American Benthological Society*, **29**, 26–40.

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. *Annual Review of Ecology and Systematics*, **29**, 59–81.

Bowman, D.M.J.S., Balch, J.K., Artaxo, P., Bond, W.J., Carlson, J.M., Cochrane, M.A., D'Antonio, C.M., DeFries, R.S., Doyle, J.C., Harrison, S.P., Johnston, F.H., Keeley, J.E., Krawchuk, M.A., Kull, C.A., Marston, J.B., Moritz, M.A., Prentice, I.C., Roos, C.I., Scott, A.C., Swetnam, T.W., van der Werf, G.R. & Pyne, S.J. (2009) Fire in the Earth System. *Science*, **324**, 481 LP – 484.

Bruder, A., Chauvet, E. & Gessner, M.O. (2011) Litter diversity , fungal decomposers and litter decomposition under simulated stream intermittency. *Fuctional Ecology*, **25**, 1269–1277.

Bruno, D., Belmar, O., Maire, A., Morel, A., Dumont, B. & Datry, T. (2019) Structural and functional responses of invertebrate communities to climate change and flow regulation in alpine catchments. *Global Change Biology*, **25**, 1612-1628.

Bruno, D., Gutiérrez-Cánovas, C., Sánchez-Fernández, D., Velasco, J. & Nilsson, C. (2016a) Impacts of environmental filters on functional redundancy in riparian vegetation. *Journal of Applied Ecology*, **53**, 846–855.

Bruno, D., Gutiérrez-Cánovas, C., Velasco, J. & Sánchez-Fernández, D. (2016b) Functional redundancy as a tool for bioassessment: A test using riparian vegetation. *Science of the Total Environment*, **566**–**567**, 1268–1276.

Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N. & Garland, T. (2008) Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 8321–8326.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimodel inference: A practical information-theoretic approach*, 2nd ed. Springer-Verlag, New York.

Burrows, R.M., Rutlidge, H., Bond, N.R., Eberhard, S.M., Auhl, A., Andersen, M.S., Valdez, D.G. & Kennard, M.J. (2017) High rates of organic carbon processing in the hyporheic zone of intermittent streams. *Scientific Reports*, **7**, 13198.

Butler, J.R.A., Radford, A., Riddington, G. & Laughton, R. (2009) Evaluating an ecosystem service provided by Atlantic salmon, sea trout and other fish species in the River Spey, Scotland: The economic impact of recreational rod fisheries. *Fisheries Research*, **96**, 259–266.

Canhoto, C., Gonçalves, A.L. & Bärlocher, F. (2016) Biology and ecological functions of aquatic hyphomycetes in a warming climate. *Fungal*

Ecology, **19**, 201–218.

Canhoto, C., Simões, S., Gonçalves, A.L., Guilhermino, L. & Bärlocher, F. (2017) Stream salinization and fungal-mediated leaf decomposition: A microcosm study. *Science of The Total Environment*, **599**–**600**, 1638–1645.

Cargill, A.S., Cummins, K.W., Hanson, B.J. & Lowry, R.R. (1985a) The role of lipids, fungi, and temperature in the nutrition of a shredder caddisfly, Clistoronia magnifica. *Freshwater Invertebrate Biology*, **4**, 64–78.

Cargill, A.S., Cummins, K.W., Hanson, B.J. & Lowry, R.R. (1985b) The role of lipids as feeding stimulants for shredding aquatic insects. *Freshwater Biology*, **15**, 455–464.

Caruso, B.S. & Haynes, J. (2011) Biophysical-Regulatory Classification and Profiling of Streams Across Management Units and Ecoregions1. *JAWRA Journal of the American Water Resources Association*, **47**, 386–407.

Chamier, A.-C. & Dixon, P.A. (1982) Pectinases in leaf degradation by aquatic hyphomycetes I: the field study. *Oecologia*, **52**, 109–115.

Chauvet, E., Cornut, J., Sridhar, K.R., Selosse, M.A. & Bärlocher, F. (2016) Beyond the water column: Aquatic hyphomycetes outside their preferred habitat. *Fungal Ecology*, **19**, 112–127.

Chessman, B.C. (2015) Relationships between lotic macroinvertebrate traits and responses to extreme drought. *Freshwater Biology*, **60**, 50–63.

Chester, E.T. & Robson, B.J. (2011) Drought refuges, spatial scale and recolonisation by invertebrates in non-perennial streams. *Freshwater Biology*, **56**, 2094–2104.

Chiu, M.-C., Leigh, C., Mazor, R., Cid, N. & Resh, V. (2017) *Chapter 5.1* -

Anthropogenic Threats to Intermittent Rivers and Ephemeral Streams. *Intermittent Rivers and Ephemeral Streams. Ecology and Management* (ed. by T. Datry), N. Bonada), and A.B.T. Boulton), pp. 433–454. Academic Press.

Chung, N. & Suberkropp, K. (2009) Effects of aquatic fungi on feeding preferences and bioenergetics of Pycnopsyche gentilis (Trichoptera: Limnephilidae). *Hydrobiologia*, **630**, 257–269.

Constantz, J., Stonestrom, D., Stewart, A.E. & Smith, T.R. (2001) Analysis of streambed temperatures in ephemeral channels to determine streamflow frequency and duration. *Water Resources Research*, **37**, 317–328.

Cornut, J., Chauvet, E., Mermillod-Blondin, F., Assemat, F. & Elger, A. (2014) Aquatic Hyphomycete Species Are Screened by the Hyporheic Zone of Woodland Streams. *Applied and Environmental Microbiology*, **80**, 1949–1960.

Cornut, J., Elger, A., Greugny, A., Bonnet, M. & Chauvet, E. (2012) Coarse particulate organic matter in the interstitial zone of three French headwater streams. *Annales de Limnologie - International Journal of Limnology*, **48**, 303–313.

Cornut, J., Elger, A., Lambrigot, D., Pierre, M. & Chauvet, E. (2010) Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. *Freshwater Biology*, **55**, 2541–2556.

Corti, R. & Drummond, T.D.L. (2011) Natural variation in immersion and emersion affects breakdown and invertebrate colonization of leaf litter in a temporary river. *Aquatic Ecology*, **73**, 537–550.

Costantini, M.L. & Rossi, L. (2010) Species diversity and decomposition in laboratory aquatic systems: The role of species interactions. *Freshwater Biology*, **55**, 2281–2295.

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.

Covich, A., Palmer, M. & Crowl, T. (1999) The Role of Benthic Invertebrate Species in Freshwater Ecosystems - Zoobenthic Species Influence Energy Flows and Nutrient Cycling. *BioScience*, **49**, 119–127.

Crabot, J., Clappe, S., Dray, S. & Datry, T. (2019) Testing the Mantel statistic with a spatially-constrained permutation procedure. *Methods in Ecology and Evolution*, **10**, 532–540.

De Crespin De Billy, V. & Usseglio-Polatera, P. (2002) Traits of brown trout prey in relation to habitat characteristics and benthic invertebrate communities. *Journal of Fish Biology*, **60**, 687–714.

Cruz-Rivera, A.E., Hay, M.E. & Ecology, S. (2008) Can Quantity Replace Quality ? Food Choice , Compensatory Feeding , and Fitness of Marine Mesograzers Published by : Ecological Society of America. *Ecology*, **81**, 201–219.

Dang, C.K., Chauvet, E. & Gessner, M.O. (2005) Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. *Ecology Letters*, **8**, 1129–1137.

Danger, M. & Chauvet, E. (2013) Elemental composition and degree of homeostasis of fungi: Are aquatic hyphomycetes more like metazoans,

bacteria or plants? *Fungal Ecology*, **6**, 453–457.

Datry, T. (2012) Benthic and hyporheic invertebrate assemblages along a flow intermittence gradient: effects of duration of dry events. *Freshwater Biology*, **57**, 563–574.

Datry, T., Bonada, N. & Boulton, A.J. (2017a) *Intermittent Rivers and Ephemeral Streams. Ecology and Management*, Academic P. ed, Elsevier Inc.

Datry, T., Bonada, N. & Heino, J. (2016) Towards understanding the organisation of metacommunities in highly dynamic ecological systems. *Oikos*, **125**, 149–159.

Datry, T., Boulton, A.J., Bonada, N., Fritz, K., Leigh, C., Sauquet, E., Tockner, K., Hugueny, B. & Dahm, C.N. (2018a) Flow intermittence and ecosystem services in rivers of the Anthropocene. *Journal of Applied Ecology*, **55**, 353–364.

Datry, T., Corti, R., Claret, C. & Philippe, M. (2011) Flow intermittence controls leaf litter breakdown in a French temporary alluvial river : the "" drying memory ."" *Aquatic Ecology*, **73**, 471–483.

Datry, T., Foulquier, A., Corti, R., von Schiller, D., Tockner, K., Mendoza-Lera, C., Clément, J.C., Gessner, M.O., Moleón, M., Stubbington, R., Gücker, B., Albariño, R., Allen, 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.J., Boëchat, I.G., Boersma, K.S., Bogan, M.T., Bonada, N., Bond, N.R., Brintrup Barría, K.C., Bruder, A., Burrows, R.M., Cancellario, T., Canhoto, C., Carlson, S.M., Cauvy-Fraunié, S., Cid, N., Danger, M., de Freitas Terra, B., De Girolamo, A.M., de La Barra, E., del Campo, R., Diaz-Villanueva, V.D., Dyer, F., Elosegi, A., Faye, E., Febria,

C., Four, B., Gafny, S., Ghate, S.D., Gómez, R., Gómez-Gener, L., Graça, M.A.S., Guareschi, S., Hoppeler, F., Hwan, J.L., Jones, J.I., Kubheka, S., Laini, A., Langhans, S.D., Leigh, C., Little, C.J., Lorenz, S., Marshall, J.C., Martín, E., McIntosh, A.R., Meyer, E.I., Miliša, M., Mlambo, M.C., Morais, M., Moya, N., Negus, P.M., Niyogi, D.K., Papatheodoulou, A., Pardo, I., Pařil, P., Pauls, S.U., Pešić, V., Polášek, M., Robinson, C.T., Rodríguez-Lozano, P., Rolls, R.J., Sánchez-Montoya, M.M., Savić, A., Shumilova, O., Sridhar, K.R., Steward, A.L., Storey, R., Taleb, A., Uzan, A., Vander Vorste, R., Waltham, N.J., Woelfle-Erskine, C., Zak, D., Zarfl, C. & Zoppini, A. (2018b) A global analysis of terrestrial plant litter dynamics in non-perennial waterways. *Nature Geoscience*, **11**, 497–503.

Datry, T., Larned, S.T., Fritz, K.M., Bogan, M.T., Wood, P.J., Meyer, E.I. & Santos, A.N. (2014a) Broad-scale patterns of invertebrate richness and community composition in temporary rivers: Effects of flow intermittence. *Ecography*, **37**, 94–104.

Datry, T., Larned, S.T. & Tockner, K. (2014b) Intermittent rivers: a challenge for freshwater ecology. *BioScience*, **64**, 229–235.

Datry, T., Singer, G., Sauquet, E., Jorda-Capdevila, D., Von Schiller, D., Magand, C., Pařil, P., Miliša, M., Acuña, V., Alves, M.H., Augeard, B., Brunke, M., Cid, N., Csabai, Z., England, J., Froebrich, J., Koundouri, P., Lamouroux, N., Martí, E., Morais, M., Munné, A., Mutz, M., Vladimir, P., Previšic, A., Reynaud, A., Robinson, C., Sadler, J., Skoulikidis, N., Terrier, B., Tockner, K., Vesely, D. & Zoppini, A. (2017b) Science and Management of Intermittent Rivers and Ephemeral Streams (SMIRES). *Research Ideas and Outcomes*, **3**, e21774.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J.,

Encinar, D., Berdugo, M., Campbell, C.D. & Singh, B.K. (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, **7**, 1–8.

Diaz, A.M., Alonso, M.L.S. & Gutierrez, M.R.V.A. (2008) Biological traits of stream macroinvertebrates from semi-arid catchment: patterns along complex environmental gradients. *Freshwater Biology*, **53**, 1–21.

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Colin Prentice, I., Garnier, E., Bönisch, G., Westoby, M., Poorter, H., Reich, P.B., Moles, A.T., Dickie, J., Gillison, A.N., Zanne, A.E., Chave, J., Joseph Wright, S., Sheremet Ev, S.N., Jactel, H., Baraloto, C., Cerabolini, B., Pierce, S., Shipley, B., Kirkup, D., Casanoves, F., Joswig, J.S., Günther, A., Falczuk, V., Rüger, N., Mahecha, M.D. & Gorné, L.D. (2016) The global spectrum of plant form and function. *Nature*, **529**, 167–171.

Dieter, D., Frindte, K., Krüger, A. & Wurzbacher, C. (2013) Preconditioning of leaves by solar radiation and anoxia affects microbial colonisation and rate of leaf mass loss in an intermittent stream. *Freshwater Biology*, **58**, 1918–1931.

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. *Environmental Research Letters*, **7**.

Duarte, S., Mora-Gómez, J. & Romanı, A.M. (2017) Responses of microbial decomposers to drought in streams may depend on the environmental context. *Environmental Microbiology Reports*, **9**, 756–765.

Duarte, S., Pascoal, C., Alves, A., Correia, A. & Cássio, F. (2010) Assessing the dynamic of microbial communities during leaf decomposition in a

low-order stream by microscopic and molecular techniques. *Microbiological Research*, **165**, 351–362.

Dufrene, M. & Legendre, P. (2011) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, **67**, 345–366.

Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B. & Norberg, J. (2003) Response diversity , ecosystem change , and resilience. *Frontiers in Ecology and the Environment*, **1**, 488–494.

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. *Science of the Total Environment, The*, **573**, 1320–1339.

Flores, L., Larrañaga, A. & Elosegi, A. (2014) Compensatory feeding of a stream detritivore alleviates the effects of poor food quality when enough food is supplied. *Freshwater Science*, **33**, 134–141.

Follstad Shah, J.J., Kominoski, J.S., Ardón, M., Dodds, W.K., Gessner, M.O., Griffiths, N.A., Hawkins, C.P., Johnson, S.L., Lecerf, A., LeRoy, C.J., Manning, D.W.P., Rosemond, A.D., Sinsabaugh, R.L., Swan, C.M., Webster, J.R. & Zeglin, L.H. (2017) Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. *Global Change Biology*, **23**, 3064–3075.

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. *Freshwater Science*, **34**, 1233–1244.

Freeman, M.C., Pringle, C.M. & Jackson, C.R. (2007) Hydrologic Connectivity and the Contribution of Stream Headwaters to Ecological Integrity at Regional Scales1. *JAWRA Journal of the American Water Resources Association*, **43**, 5–14.

Frost, P.C., Benstead, J.P., Cross, W.F., Hillebrand, H., Larson, J.H., Xenopoulos, M.A. & Yoshida, T. (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters*, **9**, 774–779.

Frost, P.C., Evans-White, M.A., Finkel, Z. V, Jensen, T.C. & Matzek, V. (2005) Are you what you eat ? Physiological in an elementally world imbalanced stoichiometry. *Oikos*, **109**, 18–28.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E.M., Steffan-Dewenter, I., Emmerson, M., Potts, S.G., Tscharntke, T., Weisser, W. & Bommarco, R. (2015) Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20142620.

Gallart, F., Cid, N., Latron, J., Llorens, P., Bonada, N., Jeuffroy, J., Jiménez-Argudo, S.M., Vega, R.M., Solà, C., Soria, M., Bardina, M., Hernández-Casahuga, A.J., Fidalgo, A., Estrela, T., Munné, A. & Prat, N. (2017) TREHS: An open-access software tool for investigating and evaluating temporary river regimes as a first step for their ecological status assessment. *Science of The Total Environment*, **607**–**608**, 519–540.

Gallart, F., Prat, N., Garca-Roger, E.M., Latron, J., Rieradevall, M., Llorens, P., Barbera, G.G., Brito, D., De Girolamo, A.M., Lo Porto, A., Buffagni, A., Erba, S., Neves, R., Nikolaidis, N.P., Perrin, J.L., Querner, E.P.,

Quinonero, J.M., Tournoud, M.G., Tzoraki, O., Skoulikidis, N., Gamez, R., Gomez, R. & Froebrich, J. (2012) A novel approach to analysing the regimes of temporary streams in relation to their controls on the composition and structure of aquatic biota. *Hydrology and Earth System Sciences*, **16**, 3165–3182.

Gana, K. & Broc, G. (2019) *Structural Equation Modeling with lavaan*, ISTE ltd and Wiley.

García-Palacios, P., Mckie, B.G., Handa, I.T., Frainer, A. & Hättenschwiler, S. (2016) The importance of litter traits and decomposers for litter decomposition: A comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology*, **30**, 819–829.

Geraldes, P., Pascoal, C. & Cássio, F. (2012) Effects of increased temperature and aquatic fungal diversity on litter decomposition. *Fungal Ecology*, **5**, 734–740.

Gessner, M.O. (2020) *Ergosterol as a measure of fungal biomass*. *Methods to Study Litter Decomposition: A Practical Guide*, pp. 231–236. Springer Netherlands, Netherlands.

Gessner, M.O. & Chauvet, E. (1993) Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502–507.

Gessner, M.O. & Chauvet, E. (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, **75**, 1807–1817.

Gessner, M.O., Chauvet, E. & Dobson, M. (1999) A Perspective on Leaf Litter Breakdown in Streams. *Oikos*, **85**, 377–384.

Gessner, M.O., Gulis, V., Kuehn, K.A., Chauvet, E. & Suberkropp, K. (2007)

Fungal Decomposers of Plant Litter in Aquatic Ecosystems. *The Mycota. Environmental and Microbial Relationships.* (ed. by D.I. Kubicek C.), pp. 301–324. Springer, Berlin, Heidelberg.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Hättenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology and Evolution*, **25**, 372–380.

Ghate, S.D. & Sridhar, K.R. (2015) Diversity of aquatic hyphomycetes in streambed sediments of temporary streamlets of Southwest India. *Fungal Ecology*, **14**, 53–61.

Gionchetta, G., Artigas, J., Arias-Real, R., Oliva, F. & Romaní, A.M. (2020) Multi-model assessment of hydrological and environmental impacts on streambed microbes in Mediterranean catchments. *Environmental Microbiology*, **00**.

Gionchetta, G., Oliva, F., Menéndez, M., Lopez, P. & Anna, L. (2019) Key role of streambed moisture and flash storms for microbial resistance and resilience to long-term drought. *Freshwater Biology*, **64**, 306–322.

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.

Gonçalves, A.L., Chauvet, E., Bärlocher, F., Graça, M.A.S. & Canhoto, C. (2014) Top-down and bottom-up control of litter decomposers in streams. *Freshwater Biology*, **59**, 2172–2182.

Gonçalves, A.L., Lírio, A.V., Graça, M.A.S. & Canhoto, C. (2016) Fungal species diversity affects leaf decomposition after drought. *International*

Review of Hydrobiology, **101**, 78–86.

Gönczöl, J., Révay, À. & Csontos, P. (2001) Effect of sample size on the detection of species and conidial numbers of aquatic hyphomycetes collected by membrane filtration. *Fundamental and Applied Limnology*, **150**, 677–691.

Graça, M.A.S. (2001) The role of invertebrates on leaf litter decomposition in streams–a review. *International Review of Hydrobiology*, **86**, 383–393.

Graça, M.A.S., Bärlocher, F. & Gessner, M.O. (2005) *Methods to Study Litter Decomposition: A Practical Guide.*, Springer Netherlands.

Graça, M.A.S., Cressa, C., Gessner, M.O., Feio, M.J., Callies, K.A. & Barrios, C. (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, **46**, 947–957.

Graça, M.A.S., Maltby, L. & Calow, P. (1993) Importance of fungi in the diet of Gammarus pulex and Asellus aquaticus I: feeding strategies. *Oecologia*, **93**, 139–144.

Grace, J.B. (2006) *Structural Equation Modeling and Natural Systems*, Cambridge University Press, New York, NY.

Grace, J.B., Anderson, T.M., Olff, H. & Scheiner, S.M. (2010) On the specification of structural equation models for ecological systems. *Ecological Monographs*, **80**, 67–87.

Granados, V., Gutiérrez-Cánovas, C., Arias-Real, R., Obrador, B., Harjung, A. & Butturini, A. (2020) The interruption of longitudinal hydrological connectivity causes delayed responses in dissolved organic matter. *Science of the Total Environment*, **713**, 136619.

Green, A.J. & Elmberg, J. (2014) Ecosystem services provided by waterbirds. *Biological Reviews*, **89**, 105–122.

Grimm, N.B. & Fisher, S.G. (1984) Exchange between interstitial and surface water: Implications for stream metabolism and nutrient cycling. *Hydrobiologia*, **111**, 219–228.

Grimmett, I.J., Shipp, K.N., Macneil, A. & Bärlocher, F. (2013) Does the growth rate hypothesis apply to aquatic hyphomycetes? *Fungal Ecology*, **6**, 493–500.

Gulis, V. (2001) Are there any substrate preferences in aquatic hyphomycetes? *Mycological Research*, **105**, 1088–1093.

Gutiérrez-Cánovas, C., Millán, A., Velasco, J., Vaughan, I.P. & Ormerod, S.J. (2013) Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Global Ecology and Biogeography*, **22**, 796–805.

Gutiérrez-Cánovas, C., Sánchez-Fernández, D., Cañedo-Argüelles, M., Millán, A., Velasco, J., Acosta, R., Fortuño, P., Otero, N., Soler, A. & Bonada, N. (2019) Do all roads lead to Rome? Exploring community trajectories in response to anthropogenic salinization and dilution of rivers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**, 20180009.

Gutiérrez-Cánovas, C., Sánchez-Fernandez, D., Velasco, J., Millán, A. & Bonada, N. (2015) Similarity in the difference: Changes in community functional features along natural and anthropogenic stress gradients. *Ecology*, **96**, 2458–2466.

Harjung, A., Perujo, N., Butturini, A., Romaní, A.M. & Sabater, F. (2019)

Responses of microbial activity in hyporheic pore water to biogeochemical changes in a drying headwater stream. *Freshwater Biology*, **64**, 735–749.

Hieber, M. & Gessner, M.O. (2002) Contribution of Stream Detrivores, Fungi, and Bacteria to Leaf Breakdown Based on Biomass Estimates. *Ecology*, **83**, 1026–1038.

Hoffman, G.E. & Schadt, E.E. (2016) variancePartition: Interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics*, **17**, 483.

Hooper, D.U., Chapin, F.S., Ewel, J.., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Shmind, B., Setäla, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Hurtado, P., Prieto, M., Aragón, G., Escudero, A. & Martínez, I. (2019) Critical predictors of functional, phylogenetic and taxonomic diversity are geographically structured in lichen epiphytic communities. *Journal of Ecology*, **107**, 2303–2316.

Iriondo, J.M., Albert, M.J. & Escudero, A. (2003) Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. *Biological Conservation*, **113**, 367–377.

Jáimez-Cuéllar, P., Vivas, S., Bonada, N., Robles, S., Mellado, A., Álvarez, M., Avilés, J., Casas, J., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Sáinz-cantero, C.E., Sánchez-ortega, A., Suárez, M.L., Toro, M., Vidal-abarca, M.R., Zamora-Muñoz, C. & Alba-Tercedor, J. (2002) Protocolo GUADALMED (PRECE). *Limnetica*, **21**, 187–204.

Jost, L. (2006) Entropy and diversity. *Oikos*, **113**, 363–375.

Kaspari, M., Donoso, D., Lucas, J.A., Zumbusch, T. & Kay, A.D. (2012) Using nutritional ecology to predict community structure: a field test in Neotropical ants. *Ecosphere*, **3**, 1–12.

Koutroulis, A.G., Papadimitriou, L. V, Grillakis, M.G., Tsanis, I.K., Warren, R. & Betts, R.A. (2019) Global water availability under high-end climate change: A vulnerability based assessment. *Global and Planetary Change*, **175**, 52–63.

Krauss, G.J., Solé, M., Krauss, G., Schlosser, D., Wesenberg, D. & Bärlocher, F. (2011) Fungi in freshwaters : ecology , physiology and biochemical potential. *FEMS Microbiology Reviews*, **35**, 620–651.

Kuehn, K.A. (2016) Lentic and lotic habitats as templets for fungal communities: Traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. *Fungal Ecology*, **19**, 135–154.

Kummu, M., Guillaume, J.H.A., de Moel, H., Eisner, S., Flörke, M., Porkka, M., Siebert, S., Veldkamp, T.I.E. & Ward, P.J. (2016) The world's road to water scarcity: shortage and stress in the 20th century and pathways towards sustainability. *Scientific Reports*, **6**, 38495.

Laforest-Lapointe, I., Paquette, A., Messier, C. & Kembel, S.W. (2017) Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature*, **546**, 145–147.

Lake, P.S. (2000) Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society*, **19**, 573–592.

Lake, P.S. (2003) Ecological effects of perturbation by drought in flowing

waters. *Freshwater Biology*, **48**, 1161–1172.

Laliberte, E. & Legendre, P. (2010) A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, **91**, 299–305.

Laliberté, E., Wells, J.A., Declerck, F., Metcalfe, D.J., Catterall, C.P., Queiroz, C., Aubin, I., Bonser, S.P., Ding, Y., Fraterrigo, J.M., McNamara, S., Morgan, J.W., Merlos, D.S., Vesk, P.A. & Mayfield, M.M. (2010) Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, **13**, 76–86.

Larned, S.T., Datry, T., Arscott, D.B. & Tockner, K. (2010) Emerging concepts in temporary-river ecology. *Freshwater Biology*, **55**, 717–738.

Lavorel, S., Storkey, J., Bardgett, R.D., De Bello, F., Berg, M.P., Roux, X.L., Moretti, M., Mulder, C., Pakeman, R.J., Díaz, S. & Harrington, R. (2013) A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. **24**, 942–948.

Leberfinger, K. & Bohman, I. (2010) Grass, mosses, algae, or leaves? Food preference among shredders from open-canopy streams. *Aquatic Ecology*, **44**, 195–203.

Leigh, C., Boulton, A.J., Courtwright, J.L., Fritz, K., May, C.L., Walker, R.H. & Datry, T. (2016) Ecological research and management of intermittent rivers: an historical review and future directions. *Freshwater Biology*, **61**, 1181–1199.

Leigh, C. & Datry, T. (2017) Drying as a primary hydrological determinant of biodiversity in river systems: a broad-scale analysis. *Ecography*, **40**, 487–499.

Lepori, F. & Hjerdt, N. (2006) Disturbance and Aquatic Biodiversity: Reconciling Contrasting Views. *BioScience*, **56**, 809–818.

Li, J., Delgado-Baquerizo, M., Wang, J.-T., Hu, H.-W., Cai, Z.-J., Zhu, Y. & Singh, B.K. (2019) Fungal richness contributes to multifunctionality in boreal forest soil. *Soil Biology and Biochemistry*, **136**, 107526.

Lytle, D.A. & Poff, N.L.R. (2004) Adaptation to natural flow regimes. *Trends in Ecology and Evolution*, **19**, 94–100.

Maire, E., Grenouillet, G., Brosse, S. & Villéger, S. (2015) How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Global Ecology and Biogeography*, **24**, 728–740.

Marcarelli, A.M., Baxter, C. V, Mineau, M.M. & Hall, R.O. (2011) Quantity and quality: Unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*, **92**, 1215–1225.

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 Reviews*, **188**, 240–248.

Mardhiah, U., Caruso, T., Gurnell, A. & Rillig, M.C. (2014) Soil Biology & Biochemistry Just a matter of time : Fungi and roots significantly and rapidly aggregate soil over four decades along the Tagliamento River , NE Italy. *Soil Biology and Biochemistry*, **75**, 133–142.

Martínez, A., Pérez, J., Molinero, J., Sagarduy, M. & Pozo, J. (2015) Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. *Science of the Total Environment*,

503–**504**, 251–257.

Marxen, J., Zoppini, A. & Wilczek, S. (2010) Microbial communities in streambed sediments recovering from desiccation. *FEMS Microbiology Ecology*, **71**, 374–386.

Mas-Martí, E., Romaní, A.M. & Muñoz, I. (2015) Consequences of warming and resource quality on the stoichiometry and nutrient cycling of a stream shredder. *PLoS ONE*, **10**, 1–21.

Mas-Martí, E., Sanpera-Calbet, I. & Muñoz, I. (2017) Bottom-up effects of streambed drying on consumer performance through changes in resource quality. *Aquatic Sciences*, **79**, 719–731.

McMullen, L.E., De Leenheer, P., Tonkin, J.D. & Lytle, D.A. (2017) High mortality and enhanced recovery: modelling the countervailing effects of disturbance on population dynamics. *Ecology Letters*, **20**, 1566–1575.

Medeiros, A.O., Pascoal, C. & Graça, M.A.S. (2009) Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology*, **54**, 142–149.

Menéndez, M., Descals, E., Riera, T. & Moya, O. (2013) Do non-native Platanus hybrida riparian plantations affect leaf litter decomposition in streams? *Hydrobiologia*, **716**, 5–20.

Menéndez, M., Descals, E., Riera, T. & Moya, O. (2012) Effect of small reservoirs on leaf litter decomposition in Mediterranean headwater streams. *Hydrobiologia*, **691**, 135–146.

Menéndez, M., Descals, E., Riera, T. & Moya, O. (2011) Leaf litter breakdown in Mediterranean streams: effect of dissolved inorganic nutrients.

Hydrobiologia, **669**, 143–155.

Moog, O. (2002) Fauna Aquatica Austriaca. A comprehensive species inventory of Austrian aquatic organisms with ecological notes, Federal Ministry of Agriculture. *Forestry, Environment and Water Management, Vienna*.

Mora-Gómez, J., Cássio, F., Pascoal, C. & Romaní, A.M. (2018) Microbial decomposition is highly sensitive to leaf litter emersion in a permanent temperate stream. *Science of the Total Environment*, **621**, 486–496.

Mora-Gómez, 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 Microbiology Ecology*, **92**, 1–13.

Mora-Gómez, J., Elosegi, A., Mas-Martí, E. & Romaní, A.M. (2015) Factors controlling seasonality in leaf-litter breakdown in a Mediterranean stream. *Freshwater Science*, **34**, 1245–1258.

Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H. & Bellwood, D.R. (2013) A functional approach reveals community responses to disturbances. *Trends in Ecology and Evolution*, **28**, 167–177.

Müller-Navarra, D.C., Brett, M.T., Liston, A.M. & Goldman, C.R. (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403**, 74–77.

Muñoz, I., Abril, M., Casas-Ruiz, J.P., Casellas, M., Gómez-Gener, L., Marcé, R., Menéndez, M., Obrador, B., Sabater, S., von Schiller, D. & Acuña, V. (2018) Does the severity of non-flow periods influence ecosystem structure and function of temporary streams? A mesocosm study.

Freshwater Biology, **63**, 613–625.

Muñoz, I. & Prat, N. (1994) Macroinvertebrate community in the lower Ebro river (NE Spain). *Hydrobiologia*, **286**, 65–78.

Murphy, J. & Riley, J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Mustonen, K.R., Mykrä, H., Louhi, P., Markkola, A., Tolkkinen, M., Huusko, A., Alioravainen, N., Lehtinen, S. & Muotka, T. (2016) Sediments and flow have mainly independent effects on multitrophic stream communities and ecosystem functions. *Ecological Applications*, **26**, 2116–2129.

Nimmo, D.G., Mac Nally, R., Cunningham, S.C., Haslem, A. & Bennett, A.F. (2015) Vive la résistance: reviving resistance for 21st century conservation. *Trends in Ecology & Evolution*, **30**, 516–523.

Odum, E.P. (1985) Trends expected in stressed ecosystems. *BioScience*, **35**, 419–422.

Paetzold, A., Bernet, J.F. & Tockner, K. (2006) Consumer-specific responses to riverine subsidy pulses in a riparian arthropod assemblage. *Freshwater Biology*, **51**, 1103–1115.

Palmer, M. & Ruhi, A. (2019) Linkages between flow regime, biota, and ecosystem processes: Implications for river restoration. *Science*, **365**.

Pascoal, C., Cássio, F., Nikolcheva, L. & Bärlocher, F. (2010) Realized fungal diversity increases functional stability of leaf litter decomposition under zinc stress. *Microbial Ecology*, **59**, 84–93.

Patrick, C.J. & Yuan, L.L. (2017) Modeled hydrologic metrics show links

between hydrology and the functional composition of stream assemblages: *Ecological Applications*, **27**, 1605–1617.

Pavoine, S., Vallet, J., Dufour, A.-B., Gachet, S. & Daniel, H. (2009) On the challenge of treating various types of variables: application for improving the measurement of functional diversity. *Oikos*, **118**, 391–402.

Pearson, C.E., Symondson, W.O.C., Clare, E.L., Ormerod, S.J., Iparraguirre Bolaños, E. & Vaughan, I.P. (2018) The effects of pastoral intensification on the feeding interactions of generalist predators in streams. *Molecular Ecology*, **27**, 590–602.

Perkins, D.M., Bailey, R.A., Dossena, M., Gamfeldt, L., Reiss, J., Trimmer, M. & Woodward, G. (2015) Higher biodiversity is required to sustain multiple ecosystem processes across temperature regimes. *Global Change Biology*, **21**, 396–406.

Petersen, R. & Cummins, K. (1974) Leaf processing in a woodland stream. *Freshwater Biology*, **4**, 343–368.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team, 2017 (2017) nlme: Linear and Nonlinear Mixed Effects Models. *R Package Version 3.1–131.*

Pinna, M. & Basset, A. (2004) Summer drought disturbance on plant detritus decomposition processes in three River Tirso (Sardinia , Italy) sub-basins. *Hydrobiologia*, **522**, 311–319.

van der Plas, F. (2019) Biodiversity and ecosystem functioning in naturally assembled communities. *Biological Reviews*, **94**, 1220–1245.

Poff, N.L.R. (1997) Landscape Filters and Species Traits: Towards

Mechanistic Understanding and Prediction in Stream Ecology. *Journal of the North American Benthological Society*, **16**, 391–409.

Poff, N.L.R., Allan, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E. & Stromberg, J.C. (1997) The Natural Flow Regime. *BioScience*, **47**, 769–784.

Pohlon, E., Fandino, A.O. & Marxen, J. (2013) Bacterial Community Composition and Extracellular Enzyme Activity in Temperate Streambed Sediment during Drying and Rewetting. *PLoS ONE*, **8**, e83365.

Pringle, C. (2003) What is hydrologic connectivity and why is it ecologically important? *Hydrological Processes*, **17**, 2685–2689.

R Development Core Team, R. (2011) *R: A Language and Environment for Statistical Computing*,.

Read, S.J., Moss, S.T. & Jones, E.B.G. (1992) *Attachment and Germination of Conidia BT - The Ecology of Aquatic Hyphomycetes*. (ed. by F. Bärlocher), pp. 135–151. Springer Berlin Heidelberg, Berlin, Heidelberg.

Reardon, J., Company, C.M. & Valley, S. (1969) Salicylate method for the quantitative determination of amonia nitrogen. *United States Patent Office*, **11**, 3–6.

Richardson, J.S., Zhang, Y. & Marczak, L.B. (2010) Resource subsidies across the land-freshwater interface and responses in recipient communities. *River Research and Applications*, **26**, 55–56.

Rolls, R.J., Heino, J., Ryder, D.S., Chessman, B.C., Growns, I.O., Thompson, R.M. & Gido, K.B. (2018) Scaling biodiversity responses to hydrological regimes. *Biological Reviews*, **93**, 971–995.

Rolls, R.J., Leigh, C. & Sheldon, F. (2012) Mechanistic effects of low-flow

hydrology on riverine ecosystems: ecological principles and consequences of alteration. *Freshwater Science*, **31**, 1163–1186.

Romaní, A.M., Fischer, H., Mille-Lindblom, C. & Tranvik, L.J. (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. *Ecology*, **87**, 2559–2569.

Rong, Q., Sridhar, K.R. & Bärlocher, F. (1995) Food selection in three leaf-shredding stream invertebrates. *Hydrobiologia*, **316**, 173–181.

Ruhí, A., Datry, T. & Sabo, J.L. (2017) Interpreting beta-diversity components over time to conserve metacommunities in highly dynamic ecosystems. *Conservation Biology*, **31**, 1459–1468.

Sanpera-Calbet, I., Acuña, V., Butturini, A., Marcé, R. & Muñoz, I. (2016) El Niño southern oscillation and seasonal drought drive riparian input dynamics in a Mediterranean stream. *Limnology and Oceanography*, **61**, 214–226.

Sanpera-Calbet, I., Ylla, I., Romaní, A.M., Sabater, S. & Muñoz, I. (2017a) Biochemical quality of basal resources in a forested stream: effects of nutrient enrichment. *Aquatic Sciences*, **79**, 99–112.

Sanpera-Calbet, I., Ylla, I., Romaní, A.M., Sabater, S. & Muñoz, I. (2017b) Drought effects on resource quality in a Mediterranean stream: fatty acids and sterols as indicators. *Limnetica*, **36**, 29–43.

Scheider, A., Jost, A., Coulon, C., Silvestre, M., Théry, S. & Ducharne, A. (2017) Global-scale river network extraction based on high-resolution topography and constrained by lithology, climate, slope, and observed drainage density. *Geophysical Research letters*, **44**, 2773–2781.

von Schiller, D., Datry, T., Corti, R., Foulquier, A., Tockner, K., Marcé, R.,

García-Baquero, G., Odriozola, I., Obrador, B., Elosegi, A., Mendoza-Lera, C., Gessner, M.O., Stubbington, R., Albariño, R., Allen, 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.S., Bogan, M.T., Bonada, N., Bond, N.R., Brintrup, K., Bruder, A., Burrows, R.M., Cancellario, T., Carlson, S.M., Cauvy-Fraunié, S., Cid, N., Danger, M., de Freitas Terra, B., Dehedin, A., De Girolamo, A.M., del Campo, R., Díaz-Villanueva, V., Duerdoth, C.P., Dyer, F., Faye, E., Febria, C., Figueroa, R., Four, B., Gafny, S., Gómez, R., Gómez-Gener, L., Graça, M.A.S., Guareschi, S., Gücker, B., Hoppeler, F., Hwan, J.L., Kubheka, S., Laini, A., Langhans, S.D., Leigh, C., Little, C.J., Lorenz, S., Marshall, J., Martín, E.J., McIntosh, A., Meyer, E.I., Miliša, M., Mlambo, M.C., Moleón, M., Morais, M., Negus, P., Niyogi, D., Papatheodoulou, A., Pardo, I., Pařil, P., Pešić, V., Piscart, C., Polášek, M., Rodríguez-Lozano, P., Rolls, R.J., Sánchez-Montoya, M.M., Savić, A., Shumilova, O., Steward, A., Taleb, A., Uzan, A., Vander Vorste, R., Waltham, N., Woelfle-Erskine, C., Zak, D., Zarfl, C. & Zoppini, A. (2019) Sediment Respiration Pulses in Intermittent Rivers and Ephemeral Streams. *Global Biogeochemical Cycles*, **33**, 1251–1263.

Schmidt-Kloiber, A. & Hering, D. (2015) www.freshwaterecology.info – An online tool that unifies , standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. *Ecological Indicators*, **53**, 271–282.

Schmitz, O. (2017) Predator and prey functional traits: Understanding the adaptive machinery driving predator-prey interactions. *F1000Research*, **6**, 1767.

Schriever, T.A., Bogan, M.T., Boersma, K.S., Cañedo-Argüelles, M., Jaeger,

K.L., Olden, J.D. & Lytle, D.A. (2015) Hydrology shapes taxonomic and functional structure of desert stream invertebrate communities. *Freshwater Science*, **34**, 399–409.

Schriever, T.A. & Lytle, D.A. (2016) Convergent diversity and trait composition in temporary streams and ponds. *Ecosphere*, **7**, 1–12.

Scott, S. & Zhang, Y. (2012) Contrasting Effects of Sand Burial and Exposure on Invertebrate Colonization of Leaves. *The American Midland Naturalist*, **167**, 68–78.

Selosse, M.-A., Vohník, M. & Chauvet, E. (2008) Out of the rivers: are some aquatic hyphomycetes plant endophytes? *New Phytologist*, **178**, 3–7.

Shipley, B. (2004) *Cause and correlation in biology: A User's guide to path analysis, structural equations and causal inference*, Cambridge University Press, Cambridge, UK.

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, del Campo, R., 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. *Global Change Biology*, **25**, 1591–1611.

Siebers, A.R., Paillex, A. & Robinson, C.T. (2019) Flow intermittency influences the trophic base, but not the overall diversity of alpine stream food webs. *Ecography*, **42**, 1523–1535.

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. *Science of The Total Environment*, **577**, 1–18.

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. *Hydrology and Earth System Sciences*, **17**, 2685–2699.

Solagaistua, L., Arroita, M., Aristi, I., Larrañaga, A. & Elosegi, A. (2016) Changes in discharge affect more surface than subsurface breakdown of organic matter in a mountain stream. *Marine and Freshwater Research*, **67**, 1826–1834.

Soria, M., Gutiérrez-Cánovas, C., Bonada, N., Acosta, R., Rodríguez-Lozano, P., Fortuño, P., Burgazzi, G., Vinyoles, D., Gallart, F., Latron, J., Llorens, P., Prat, N. & Cid, N. (2020) Natural disturbances can produce misleading bioassessment results: Identifying metrics to detect

anthropogenic impacts in intermittent rivers. *Journal of Applied Ecology*, **57**, 283–295.

Soria, M., Leigh, C., Datry, T., Bini, L.M. & Bonada, N. (2017) Biodiversity in perennial and intermittent rivers: a meta-analysis. *Oikos*, **126**, 1078–1089.

Sponseller, R.A., Heffernan, J.B. & Fisher, S.G. (2013) On the multiple ecological roles of water in river networks. *Ecosphere*, **4**, 17.

Sridhar, K.R. (2009) *Fungi in the tree canopy– An appraisal*, Applied My. (ed. by M. Rai) and P. Bridge) CAB International, UK.

Sridhar, K.R., Duarte, S., Cássio, F. & Pascoal, C. (2009) The Role of Early Fungal Colonizers in Leaf-Litter Decomposition in Portuguese Streams Impacted by Agricultural Runoff. *International Review of Hydrobiology*, **4**, 399–409.

Sterner, R.W. & Elser, J.J. (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*, Princeton University Press.

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. *Frontiers in Ecology and the Environment*, **10**, 202–209.

Storey, R.G., Fulthorpe, R.R. & Williams, D.D. (1999) Perspectives and predictions on the microbial ecology of the hyporheic zone. *Freshwater Biology*, **41**, 119–130.

Stout, R.J. (1989) Effects of Condensed Tannins on Leaf Processing in Mid-Latitude and Tropical Streams: A Theoretical Approach. *Canadian Journal of Fisheries and Aquatic Sciences*, **46**, 1097–1106.

Stubbington, R. (2012) The hyporheic zone as an invertebrate refuge : A

review of variability in space , time , taxa and behaviour. *Marine and Freshwater Research*, **63**, 293–311.

Stubbington, R., Chadd, R., Cid, N., Csabai, Z., Miliša, M., Morais, M., Munné, A., Pařil, P., Pešić, V., Tziortzis, I., Verdonschot, R.C.M. & Datry, T. (2018) Biomonitoring of intermittent rivers and ephemeral streams in Europe: Current practice and priorities to enhance ecological status assessments. *Science of the Total Environment*, **618**, 1096–1113.

Stubbington, R. & Datry, T. (2013) The macroinvertebrate seedbank promotes community persistence in temporary rivers across climate zones. *Freshwater Biology*, **58**, 1202–1220.

Stubbington, R., Gunn, J., Little, S., Worrall, T.P. & Wood, P.J. (2016) Macroinvertebrate seedbank composition in relation to antecedent duration of drying and multiple wet-dry cycles in a temporary stream. *Freshwater Biology*, **61**, 1293–1307.

Suberkropp, K. & Arsuffi, T.L. (1984) Degradation, growth, and changes in palatability of leaves colonized by six aquatic hyphomycete species. *Mycologia*, **76**, 398–407.

Suberkropp, K., Arsuffi, T.L. & Anderson, J.P. (1983) Comparison of degradative ability , enzymatic activity , and palatability of aquatic hyphomycetes grown on leaf litter. *Applied and Environmental Microbiology*, **46**, 237–244.

Suberkropp, K., Gulis, V., Rosemond, A.D. & Benstead, J.P. (2010) Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream : Results of a 5-year continuous enrichment. *Limnology and oceanography*, **55**, 149–160.

Suding, K.N., Lavorel, S., Chapin, F.S., Cornelissen, J.H.C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. & Navas, M.L. (2008) Scaling environmental change through the community-level: A trait-based response-and-effect framework for plants. *Global Change Biology*, **14**, 1125–1140.

Tachet, H., Richoux, P., Bournaud, M. & Usseglio-Polatera, P. (2002) *Invertébrés d'Eau Douce (2nd corrected impression)*, CNRS éditions, Paris, France.

Tonkin, J.D., Bogan, M.T., Bonada, N., Rios-Touma, B. & Lytle, D.A. (2017) Seasonality and predictability shape temporal species diversity. *Ecology*, **98**, 1201–1216.

Tonkin, J.D., Merritt, D.M., Olden, J.D., Reynolds, L. V. & Lytle, D.A. (2018) Flow regime alteration degrades ecological networks in riparian ecosystems. *Nature Ecology and Evolution*, **2**, 86–93.

Torres-Ruíz, M. & Wehr, J.D. (2010) Changes in the nutritional quality of decaying leaf litter in a stream based on fatty acid content. *Hydrobiologia*, **651**, 265–278.

Torres-Ruíz, M., Wehr, J.D. & Perrone, A.A. (2007) Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society*, **26**, 509–522.

Trzcinski, M.K., Srivastava, D.S., Corbara, B., Dézerald, O., Leroy, C., Carrias, J.F., Dejean, A. & Céréghino, R. (2016) The effects of food web structure on ecosystem function exceeds those of precipitation. *Journal of Animal Ecology*, **85**, 1147–1160.

Vannote, R.L., Minshall, G.W., Cummis, K.W., Sedell, J.R. & Cushing, C.E. (1980) The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Vázquez, E., Romaní, A.M., Sabater, F. & Butturini, A. (2007) Effects of the Dry–Wet Hydrological Shift on Dissolved Organic Carbon Dynamics and Fate Across Stream–Riparian Interface in a Mediterranean Catchment. *Ecosystems*, **10**, 239–251.

Veech, J.A. (2012) Significance testing in ecological null models. *Theoretical Ecology*, **5**, 611–616.

Villéger, S., Mason, N.W.H. & Mouillot, D. (2008) New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**, 2290–2301.

Vörösmarty, C.J. & Sahagian, D. (2000) Anthropogenic Disturbance of the Terrestrial Water Cycle. *BioScience*, **50**, 753–765.

Vorste, R. Vander, Corti, R., Sagouis, A. & Datry, T. (2016) Invertebrate communities in gravel-bed, braided rivers are highly resilient to flow intermittence. *Freshwater Science*, **35**, 164–177.

Wang, Y., Naumann, U., Wright, S.T. & Warton, D.I. (2012) Mvabund- an R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution*, **3**, 471–474.

Ward, J. V (1989) The Four-Dimensional Nature of Lotic Ecosystems. *Journal of the North American Benthological Society*, **8**, 2–8.

Warkentin, M., Freese, H.M., Karsten, U. & Schumann, R. (2007) New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor

spots. *Applied and Environmental Microbiology*, **73**, 6722–6729.

Williams, D.D. (2006) *The Biology of Temporary Waters*, Oxford University Press, Oxford.

Woodward, G. (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. *Freshwater Biology*, **54**, 2171–2187.

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A.S., Fleituch, T., Lacoursière, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.M. & Chauvet, E. (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, **336**, 1438–1440.

Zöllner, N. & Kirsch, K. (1962) Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. *Zeitschrift für die gesamte experimentelle Medizin*, **135**, 545–561.

Zoppini, A., Ademollo, N., Amal, S., Casella, P., Patrolecco, L. & Polesello, S. (2014) Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. *Science of the Total Environment*, **484**, 74–83.

Zuur, A.F. (2009) *Violation of Independence – Part I. Mixed Effects Models and Extensions in Ecology with R*, pp. 143–160.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010) A protocol for data exploration

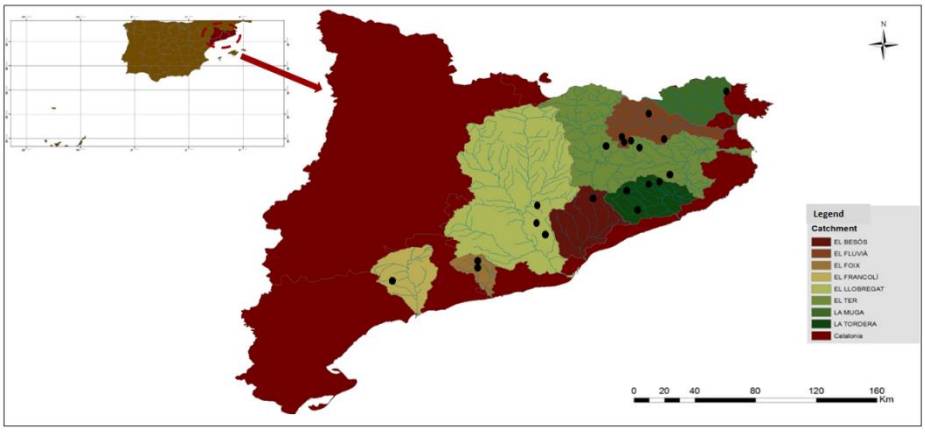
to avoid common statistical problems. *Methods in Ecology and Evolution*, **1**, 3–14.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed effects models and extensions in ecology with R*, Springer. Springer-Verlag New York., New York, NY.

ANNEX (Supplementary materials)

CHAPTER 1

S1-Study site



S1-Figure 1. Map of the study area showing the eight basins and the sampling sites.

S1-Variance Inflation Factor (VIF)

S1-Table 1. Variance Inflation Factor (VIF) values for decay rates and fungal biomass included in the final models for surface zones.

Variables	VIF
Total non-flow days (TNF)	1.05
Conductivity	1.01
Soluble reactive phosphorus (SRP)	1.01

S1-Table 2. Variance Inflation Factor (VIF) values for decay rates included in the final model for subsurface zone.

Variables	VIF
Total non-flow days (TNF)	1.43
Fine sand	1.22
Moisture content	1.51
C:N ratios of the sediment	1.15

S1-Table 3. Variance Inflation Factor (VIF) values for fungal biomass included in the final model for subsurface zone.

Variables	VIF
Total non-flow days (TNF)	1.47
Coarse sand	1.52
Moisture content	1.42
C:N ratios of the sediment	1.47

S1-Hydrology

S1-Table 4. Hydrology based on total non-flow days (TNF) of each stream site. * means that in these streams the sensor were lost or out of service. The total days measured in the study lasted between September 2016 and September 2017.

Site	TNF Time 90	TNF Time 360
1	0	69
2	40	150
3	79	198
4	7	163
5	65	*
6	4	68
7	9	80
8	0	0
9	23	166
10	99	322
11	56	*
12	0	102
13	26	158
14	0	0
15	21	36
16	0	0
17	0	116
18	0	0
19	0	0
20	0	109

S1-Table 5. Physicochemical (Mean ± SEM) characteristics of running water during the study period (n=3). * means no data for these sites.

	SRP (ppm)	DIN (ppm)	T (º C)	pH	Conductivity (µS/cm)	Dissolved oxygen (mg /l)
1	0.014 (±0.007)	0.424 (±0.176)	12.7 (±1.4)	8.1 (±0.1)	314.3 (±23.3)	6.3 (±1.7)
2	0.026 (±0.001)	1.808 (±0.792)	7.4 (±1.4)	9.2 (±0.1)	266.0 (±22.4)	6.5 (±1.3)
3	1.727 (±0.011)	6.174 (±3.909)	8.9 (±1.3)	8.8 (±0.1)	272.6 (±21.5)	5.6 (±1.2)
4	0.079 (±0.001)	2.554 (±2.518)	7.2 (±1.2)	9.1 (±0.1)	234.8 (±20.6)	6.5 (±1.3)
5	0.009 (±0.003)	0.849 (±0.156)	10.4 (±1.3)	9.1 (±0.1)	255.3 (±24.2)	6.8 (±1.5)
6	0.017 (±0.004)	4.341 (±2.374)	13.7 (±1.8)	8.9 (±0.1)	713.7 (±97.6)	6.8 (±0.9)
7	0.014 (±0.001)	1.664 (±0.678)	14.2 (±1.6)	8.1 (±0.1)	827.0 (±96.4)	4.9 (±1.5)
8	0.014 (±0.007)	0.734 (±0.158)	12.1 (±1.4)	8.8 (±0.3)	426.8 (±65.9)	7.1 (±0.8)
9	0.008 (±0.002)	0.462 (±0.278)	3.9 (±0.78)	9.1 (±0.1)	330.6 (±22.4)	9.2 (±1.6)
10	*	*	*	*	*	*
11	*	*	*	*	*	*
12	0.032 (±0.012)	0.693 (±0.533)	13.0 (±3.8)	8.5 (±0.7)	223.9 (±59.3)	6.2 (±0.6)
13	0.008 (±0.002)	0.538 (±0.134)	7.4 (±2.7)	6.8 (±2.1)	492.7 (±19.4)	8.3 (±0.3)

14	0.010(±0.003)	2.431(±0.494)	10.0 (±3.5)	9.1 (±0.1)	359.4 (±24.6)	7.4 (±1.3)
15	0.164 (±0.088)	4.807 (±3.363)	7.9 (±2.1)	8.1 (±0.2)	542.4 (±11.5)	5.3 (±1.2)
16	0.022 (±0.007)	6.141 (±0.242)	12.1 (±1.7)	8.9 (±0.1)	439.4 (±29.9)	6.9 (±0.8)
17	0.009 (±0.001)	5.257 (±2.413)	14.4 (±1.8)	8.5 (±0.1)	306.8 (±25.6)	8.4 (±1.8)
18	0.020 (±0.033)	0.658 (±0.085)	11.7 (±1.5)	8.5 (±0.0)	164.6 (±12.8)	6.8 (±1.0)
19	0.027 (±0.012)	3.244 (±1.184)	11.8 (±1.4)	8.6 (±0.1)	249.9 (±22.6)	6.3 (±1.1)
20	0.022(±0.001)	1.974 (±1.764)	13.0 (±3.4)	8.5 (±0.0)	227.0 (±13.7)	7.2 (±1.5)

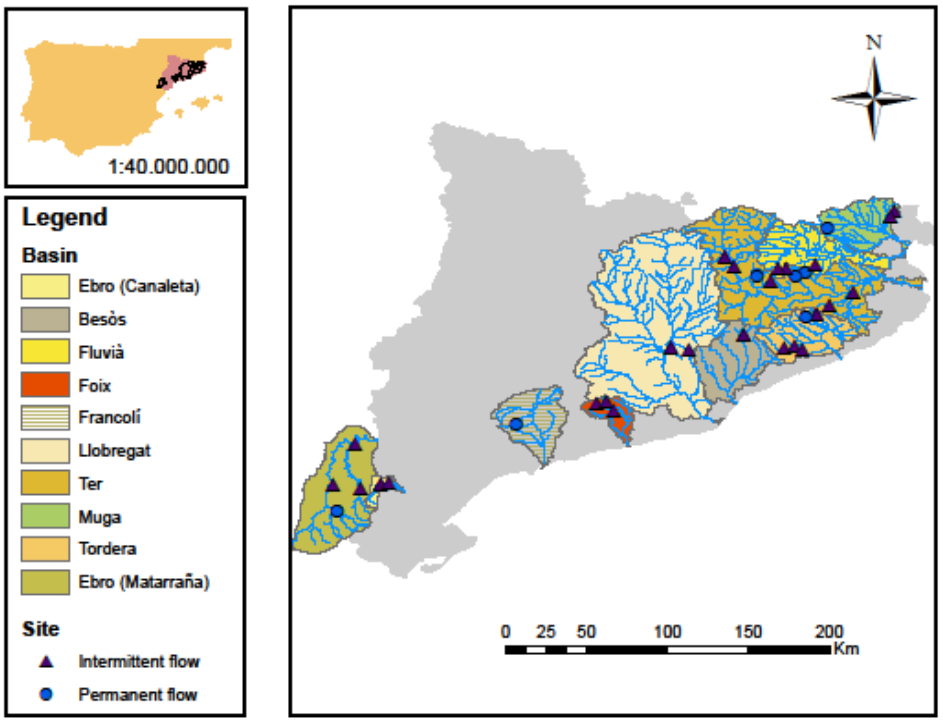
S1- Table 6. Sediment characterization (n=3). * means no data for these sites.

	Water content (%)	Sediment grain size (%)					C:N
		clay (4µm)	Silt (4-63µm)	fine sand (63-250µm)	coarse sand (250-2500µm)	gravel (>2500µm)	
1	25	0.07	0.28	0.04	72.61	27.00	18.2 (±2.2)
2	16	4.65	27.39	29.78	23.55	14.63	32.7 (±3.8)
3	12	15.03	39.59	4.57	11.47	29.34	23.2 (±0.3)
4	8	19.31	48.21	0.15	9.72	22.61	29.2 (±1.9)

5	2	0.04	0.15	0.16	76.13	23.52	142.9 (±25.8)
6	19	10.67	30.21	2.05	13.99	43.07	77.7 (±4.6)
7	84	1.05	7.82	18.40	72.73	0.00	27.9 (±0.9)
8	39	4.91	17.25	2.67	13.21	61.95	186.8 (±36.3)
9	7	1.69	13.23	17.91	67.17	0.00	44.4 (±0.6)
10	2	0.96	5.73	5.85	52.85	34.61	41.5 (±0.7)
11	5	2.12	16.33	13.71	53.26	14.59	41.4 (±6.4)
12	23	0.50	3.13	0.50	61.04	34.83	14.7 (±0.6)
13	7	6.69	27.29	14.90	19.28	31.84	86.3 (± 1.5)
14	28	1.30	6.32	14.32	72.74	5.32	65.5 (±2.4)
15	6	0.25	1.12	1.49	83.57	13.57	9.1 (±0.2)
16	39	3.92	26.59	32.98	22.39	14.11	102.1 (±6.4)
17	17	0.59	6.18	4.61	76.89	11.74	13.7 (±0.9)
18	18	0.00	0.00	0.00	56.22	43.78	*
19	25	0.08	0.31	0.86	98.75	0.00	*
20	21	0.15	0.98	0.75	69.64	28.48	15.1 (±0.8)

CHAPTER 2

S2-Study site



S2-Figure 2. Map of the study area showing the nine basins and the sampling sites. Circles: sites with permanent flow; triangles: sites with intermittent flow.

S2-Table 7. Characterization of the studied sites. The percentages of land-use cover (urban, agriculture and natural) refer to a buffer area of 1 km around each sampling point.

Site	Altitude (m.a.s.l)	Order	Basin	Precipitation (mm)	Catchment area (km^2)	Urban	Agriculture	Natural
1	258	4	Llobregat	634	23.96	0	0	100
2	468	4	Llobregat	692	8.68	0	0	100
3	430	3	Besós	788	6.50	2.17	0.82	97.01
4	540	4	Besós	748	19.35	0	1.9	98.1
5	620	2	Foix	568	1.65	0	27.12	72.88
6	390	3	Foix	574	1.89	0.00	96.44	3.56
7	330	4	Foix	590	26.87	0.00	100.00	0.00
8	170	3	Tordera	908	12.44	0.79	3.27	95.94
9	655	4	Tordera	875	49.06	0.00	0.00	100.00
10	110	4	Tordera	908	12.44	0	0	100
11	110	3	Tordera	865	49.06	0.00	0.59	99.41
12	240	4	Tordera	874	49.06	0.49	0.74	98.77
13	385	2	Ter	1013	13.41	0	1.8	98.2
14	530	2	Ter	963	13.32	0	16.67	83.33
15	920	2	Ter	1063	3.71	0	29.03	70.97

16	385	3	Ter	1038	9.17	0.00	0.89	99.11
17	630	4	Ter	953	30.99	1.44	0.03	98.53
18	632	4	Ter	953	30.99	0	2.73	97.27
19	81	4	Ter	815	7.71	0	6.25	39.75
20	526	3	Ter	963	13.32	0.00	0.58	99.42
21	140	3	Ter	816	7.71	0.00	78.49	21.51
22	605	4	Francolí	470	28.93	0.00	14.13	85.87
23	266	3	Fluvià	997	13.78	0	18.42	81.58
24	475	4	Fluvià	475	965.43	0.05	2.27	97.69
25	258	4	Muga	1093	44.93	0	0.54	99.46
26	100	3	Muga	842	13.53	0.00	99.49	0.51
27	88	4	Muga	868	48.69	0	1.99	98.01
28	415	4	Ebro	638	116.46	0	98.99	1.01
29	170	4	Ebro	540	1035.67	0.00	30.49	69.51
30	492	3	Ebro	667	207.51	0.18	98.16	1.66
31	85	3	Ebro	709	29.17	0.00	1.75	98.25
32	225	4	Ebro	561	70.51	0.00	96.94	3.06
33	487	3	Ebro	427	34.99	0.00	95.28	4.72

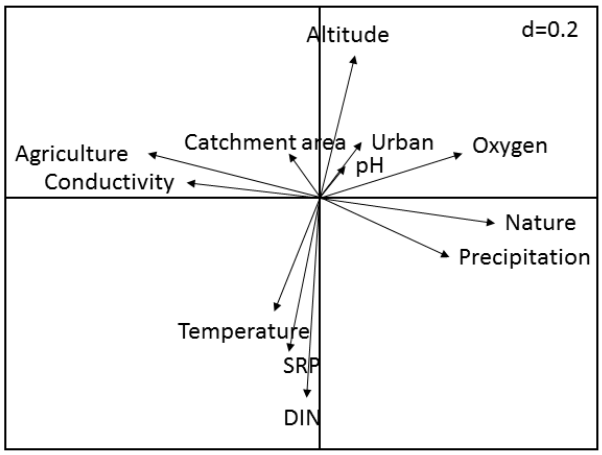
S2- Environmental characterization

For each stream location, to characterize water chemistry, we performed three sampling campaigns: the first campaign occurred during January 2016, the second campaign occurred during September/October 2016 and the third campaign occurred during February 2017. At each sampling time and location, when surface water was present, we measured the water electrical conductivity, water temperature, pH and dissolved oxygen (±1 µs cm^{-1}, ±0.1 °C, ±0.005 pH and ±0.1 mg L^{-1}, respectively) using a portable probe (YSI Professional Plus Multiparameter Instrument, USA). Furthermore, we took three replicate water samples per location to characterize the nutrient concentrations: nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and soluble reactive phosphorus (SRP). Prior to analysis, water samples were filtered through pre-combusted glass fibre filters in the field (0.7 µm pore size; Whatman GF/F, Germany) and then transported to the laboratory under cooled conditions. In the laboratory, we stored the water samples at 4 ºC in darkness, until analysis (between 24 and 48 hours).

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

S2-Table 8. Pearson correlations (*r*) between the first two non-hydrological environmental PCA axes and the original climatic, geomorphological, land-use and water chemistry variables. Axis 1 explained 27.31%; axis 2 explained 14.90%. Correlations with $r \geq |0.50|$ are in bold.

Environmental variable	Axis 1	Axis 2
Water temperature (ºC)	-0.10	-0.48
pH	0.17	0.12
Conductivity (µS cm-1)	**-0.71**	0.07
Oxygen (mg/l)	**0.73**	0.19
SRP (ppm)	-0.06	**-0.61**
DIN (ppm)	-0.08	**-0.80**
Altitude (m.a.s.l)	0.16	**0.58**
Order	0.01	0.15
Annual precipitation (mm)	**0.68**	-0.22
Catchment area (km^2)	-0.11	0.18
Urban land cover (%)	0.28	0.20
Agriculture land cover (%)	**-0.89**	0.16
Natural land cover (%)	**0.90**	-0.10



S2-Figure 3. Loadings of original variables in the two first axis of the non-hydrological PCA.

S2-Response traits

S2-Table 9. Response traits used in this study.

Response trait	Rationale	References
Short lifespan	Confers reduced time and energy to reach adult stage and reproduce earlier	(Diaz *et al.*, 2008; Chessman, 2015)
Multivoltinism	Provides increased capacity to increase the population and recover after disturbances	(Diaz *et al.*, 2008; Belmar *et al.*, 2019; Bruno *et al.*, 2019)
Ovoviparity and terrestrial reproduction	Gives more independence from the aquatic environmental conditions	(Diaz *et al.*, 2008; Belmar *et al.*, 2019; Bruno *et al.*, 2019)
Aerial respiration	Spiracle and hydrostatic vesicle modes allow low dissolved oxygen concentrations to be tolerated, which may occur during flow cessation, through aerial breathing.	(Diaz *et al.,* 2008; Chessman, 2015; Aspin *et al.,* 2019)

S2-Functional space

To derive trait-based metrics, we first built both response and trophic spaces through Principal Coordinate Analyses (PCoA) to represent response and trophic traits along independent axes of variation. Each space was based on dissimilarity matrices that were calculated using a fuzzy-coding adapted Gower index (Pavoine *et al.*, 2009) from response and trophic trait matrices. For both response and trophic spaces, we selected spaces of four axes following (Maire *et al.*, 2015) because they adequately represented the original Gower dissimilarity matrices (response space represented 65.5% of the original variance; mean squared error: 0.02; trophic space represented 89.9% of the original variance, mean squared error: 0.01). Then, we calculated response and trophic functional richness as the hypervolumes enclosing the functional space filled by the community. Response and trophic functional dispersion were calculated as the abundance-weighted functional dispersion of each community with respect to the community centroid in the response or trophic space (Laliberte & Legendre, 2010). In addition, we calculated functional redundancy (FR) as the average abundance of species per functional group (Laliberté *et al.*, 2010).

Supplementary results for response and trophic spaces

Response-trait space had four dimensions explained 65.5% of the original trait variation when combined (Axis 1: 23.77%, Axis 2: 16.74%). Response axis 1 was positively associated with organisms that presented a lifespan less than one year (r=0.88) and one generation per year (r=0.59) and negatively associated with organisms that presented a lifespan greater than one year (r=-0.88) and less than one generation per year (r=-0.71).

Response axis 2 was positively linked with organisms that reproduced through isolated eggs (r=0.54) and breathed through spiracles (r=0.7), and negatively linked with organisms that reproduced thought clutches that were cemented or fixes (r=-0.78) and breathed through their tegument (r=-0.52). Positive values on response axis 3 were linked with organism that had a reproduction though isolated eggs or cemented (r=0.77). Negative values of response axis 4 are linked with organisms that presented more than one generation per year (r=-0.52), and their respiration was thought gills (r=-0.7) **(S2-Table 10)**.

Trophic space had four dimensions that explained 89.9% of the original trophic trait variation when combined (Axis 1: 42.31%, Axis 2: 21.48%). Trophic axis 1 was positively related to grazers (r=0.61) and gathers (r=0.52), and it was negatively related to predators (r=-0.96). Trophic axis 2 is positively related with shredders (r=0.91) and it is negatively related with grazers (r=-0.53). Trophic axis 3 was positively related to grazers (r=0.53) and negatively related to gathers (r=-0.76). Trophic axis 4 was positively related to filter feeders (r=0.89) **(S2-Table 11)**.

S2-Table 10. Pearson correlation coefficients between response space axes and original response trait categories. Correlation coefficients $r \leq |0.50|$ are in bold. c1, c2 and c3 is number of generation per year.

Code	Trait	Category	Axis 1	Axis 2	Axis 3	Axis 4
b1	Lifespan	less 1 yr	**0.88**	0.16	0.29	-0.27
b2	Lifespan	greater 1 yr	**-0.88**	-0.16	-0.29	0.27
c1	Number of generations	less 1 yr	**-0.71**	-0.12	0.22	0.24
c2	Number of generations	1 yr	**0.59**	-0.26	0.04	0.25
c3	Number of generations	greater 1 yr	0.02	0.41	-0.26	**-0.52**
e1	Reproduction	ovoviviparity	-0.12	0.20	0.03	-0.32
e2	Reproduction	isolated eggs, free	-0.02	**0.54**	0.01	0.14
e3	Reproduction	isolated eggs, cemented	-0.11	0.17	**0.77**	0.14
e4	Reproduction	clutches, cemented or fixed	0.27	**-0.78**	-0.47	-0.16
e5	Reproduction	clutches, free	0.10	0.26	-0.06	0.03
e6	Reproduction	clutches, in vegetation	-0.16	0.17	0.04	0.12
e7	Reproduction	clutches, terrestrial	-0.10	0.30	-0.17	0.12
e8	Reproduction	asexual reproduction	-0.10	-0.07	-0.15	0.04
j1	Respiration	tegument	0.32	**-0.52**	0.40	0.44
j2	Respiration	gill	-0.50	-0.12	0.30	**-0.70**
j3	Respiration	plastron	-0.19	-0.05	-0.41	0.10
j4	Respiration	spiracle	0.28	**0.70**	-0.46	0.19
j5	Respiration	hydrostatic vesicle	-0.04	0.03	-0.25	0.06
		% explained variance	23.77	16.74	14.33	10.72

S2-Table 11. Pearson correlation coefficients between trophic space axes and original trophic trait categories. Correlation coefficients $r \leq |0.50|$ are in bold.

Code	Category	Axis 1	Axis 2	Axis 3	Axis 4
GRA	Grazers	**0.61**	**-0.53**	**0.53**	-0.08
MIN	Omnivores	-0.02	0.05	0.14	-0.01
XYL	Omnivores	-0.06	0.17	0.09	0.05
SHR	Shredders	0.25	**0.91**	0.23	-0.07
GAT	Gathers	**0.52**	-0.13	**-0.76**	-0.30
AFF	Filter feeders	0.06	-0.12	-0.07	0.18
PFF	Filter feeders	-0.02	-0.04	-0.23	**0.89**
PRED	Predators	**-0.96**	-0.13	0.06	-0.19
PAR	Omnivores	0.04	-0.08	-0.16	-0.12
OTHER	Omnivores	0.26	0.05	0.26	-0.04
	% explained variance	42.31	21.48	16.10	10.07

S2-Linear Regression Models

S2-Table 12. Twelve competing models representing the different ecological filters: intermittence regime and aquatic status (hydrological filters) and the model that included only environmental variability (non-hydrological filter). Predictive metrics: ENV: first non-hydrological environmental PCA axis (see details in **S2-Environmental characterization**), TNF: total non-flow days; NFP: number of non-flow periods; NF: duration of the last non-flow period; RE: duration of last rewetting event. Ecological mechanisms: ENV, non-hydrological environmental filter; IR, intermittence regime; IS, intermittence status. Grey cells indicate the flow-intermittence metrics included in a particular model.

	ENV	Flow intermittence metrics				Ecological mechanism
		TNF	NFP	NF	RE	
Mod0	■					ENV
Mod1	■	■				IR+ENV
Mod2	■		■			IR+ENV
Mod3	■	■	■			IR+ENV
Mod4	■			■		IS +ENV
Mod5	■				■	IS+ENV
Mod6	■			■	■	IS+ENV
Mod7	■	■	■		■	IR+IS+ENV
Mod8	■	■			■	IR+IS+ENV
Mod9	■		■	■		IR+IS+ENV
Mod10	■		■		■	IR+IS+ENV
Mod11	■		■	■	■	IR+IS+ENV

S2-Table 13: **Pearson correlation coefficients among predictive metrics.** Correlation coefficients $r \leq |0.70|$ are in bold.

	TNF	NFP	NF	RE
NFP	0.61			
NF	**0.72**	0.26		
RE	-0.54	-0.53	-0.63	
ENV	-0.14	-0.18	-0.08	0.18

S2- Supplementary results

S2-Table 14. Variance partitioning for predictors of the most explanatory models for community metrics (see details in Table 6 and Figure 16).

			Non-hydrological	Intermittence regime		Intermittence status	
	Community metric	Mod	ENV	TNF	NFP	NF	RE
TD	Ric	1	20.9	23.3			
	Ric	8	18.5	7.8			21.2
	Ric	5	19.7				21.2
	Sha	0	21.3				
	Sha	5	17.3				8.2
	Abun	1	0.2	27.5			
FD	R-FRic	5	9.2				23.9
	R-FRic	10	7.8		22.6		7.8
	R-FRic	2	9.4		22.6		
	R-FRic	8	8.6	4.3			23.9
	R-FRic	9	9		16.5	10.2	

R-FRic	1	10.8	19.2			
R-FRic	3	9	3.5	22.6		
T-FRic	6	22.4			0.1	20.4
T-FRic	5	23.7				13.5
T-FRic	0	25.3				
R-FDis	2	19.4		17.1		
T-FDis	1	24.7	17.4			
T-FDis	2	23.3	18.1			
T-FDis	5	23.4				17.1
T-FDis	8	22.3	5.4			17.1
T-FDis	3	22.6	4.1	18.1		
T-FDis	10	21.3		18.1		4.9
T-FDis	9	22.8		13.1	8.4	
FR	1	0.6	25.4			

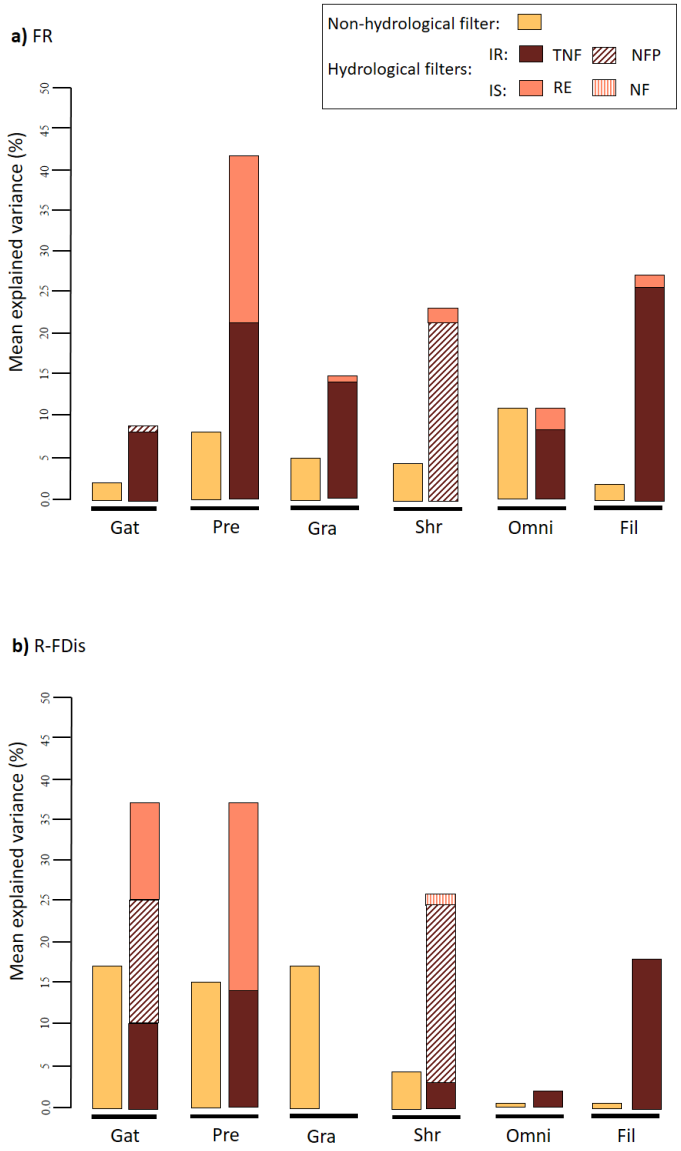
S2-Table 15. Null model results for trait-based community metrics, based on direct and indirect tests. For each model term, Standarized Effect Size (SES), *p*-value (P) and t-value are showed (only in Indirect test). See **Table 6 and Figure 16** of the main text for abbreviations.

Community metrics		Direct test		Indirect test	
	Terms	SES	P	t value	P
R-FRic	Intercept	-1.27	0.20		
R-FRic	ENV	-0.93	0.36		
R-FRic	RE	0.87	0.38	-27.56	0.00
T-FRic	Intercept	-1.57	0.13		
T-FRic	ENV	1.20	0.23		
T-FRic	NF	1.39	0.16		
T-FRic	RE	1.21	0.23	-35.63	0.00
R-FDis	Intercept	-1.25	0.21		
R-FDis	ENV	1.37	0.16		
R-FDis	NFP	-1.18	0.23	37.25	0.00
T-FDis	Intercept	-1.49	0.15		
T-FDis	ENV	2.37	0.03		
T-FDis	TNF	-1.77	0.10	56.09	0.00
FR	Intercept	0.16	0.73		
FR	ENV	-0.42	0.59		
FR	TNF	1.13	0.21	-35.63	0.00

S2-Table 16. Variance partitioning for predictors of the most explanatory models for functional redundancy (FR) and response diversity (T-FDis) of trophic groups (see details in Table 6). See **Table 7 and Figure 16** of the main text for abbreviations.

			Non-hydrological	Intermittence regime		Intermittence status	
	Trophic group	Mod	ENV	TNF	NFP	NF	RE
FR	Gat	0	3.4				
	Gat	1	2.2	7			
	Gat	6	3.1	10.6	0.01		
	Pre	1	9.1	30.8			
	Pre	5	7.7	13.6			20.5
	Gra	1	3.4	12.7			
	Gra	5	4.5	16.2			0.1
	Gra	0	5.5				
	Shr	4	4.1		20.8		
	Shr	9	3.4		20.8		2.8
	Omni	1	10.9	6.8			
	Omni	0	8.4				
	Omni	2	11.1				3.7
	Fil	1	1.9	27.9			
	Fil	5	2.4	27.3			2.7
R-FDis	Gat	1	17.9	16.3			
	Gat	4	17.1		15.3		
	Gat	2	17.3				13.3
	Gat	6	16.3	4.3	15.3		

	Pre	5	14.7	6.5			23.5
	Pre	1	17.2	22.5			
	Pre	2	15.7				23.5
	Gra	0	17.7				
	Shr	4	4.5		23.5		
	Shr	6	4.8	4.5	23.5		
	Shr	10	4.7		26.6	0.3	
	Omni	0	0.9				
	Omni	1	0.5	3.4			
	Fil	1	0.5	18.2			



S2-Figure 4. Barplot showing mean explained variance (percentage, %) of hydrological (intermittence regime and status) and non-hydrological filters for functional redundancy (FR) (a) and response diversity (R-FDis) of trophic. See **Table 7 and Figure 16** of the main text for abbreviations.

CHAPTER 3

S3-Study area

S3-Table 17. Geographical and basin characterization of the studied sites. The percentages of land use cover refer to a buffer area of 1 km around each sampling point.

Site	Altitude (m.a.s.l)	Order	Basin	Prec.	Catch.	Land Use Cover (%)		
						Urban	Agric.	Nat.
1	489	3	Tordera	911	12.4	0	0.2	99.8
2	655	4	Tordera	875	49.1	0	0	100
3	170	3	Tordera	908	12.4	0.7	3.3	95.9
4	430	3	Besós	788	6.5	2.2	0.8	97.0
5	100	3	Muga	842	13.5	0	99.5	0.5
6	385	3	Ter	1038	9.1	0	0.9	99.1
7	630	4	Ter	953	30.9	1.4	0.03	98.5
8	526	3	Ter	963	13.3	0	0.6	99.4
9	140	3	Ter	816	7.7	0	78.5	21.5
10	605	4	Francolí	470	28.9	0	14.1	85.9
11	330	4	Foix	590	26.8	0	100	0
12	475	4	Fluviá	475	965.4	0	2.3	97.7
13	219	4	Fluviá	998	13.7	0	34.4	65.6
14	484	4	Fluviá	1026	15.7	0	1.1	49.3
15	325	3	Llobregat	732	10.9	2.1	0.9	96.9

Prec. = annual precipitation (mm); Catch. = catchment area (Km^2); Agric. = extensive agriculture; Nat. = nature that include: forest (broad-leaved forest. mixed forest and coniferous forest), scrubland and grasslands.

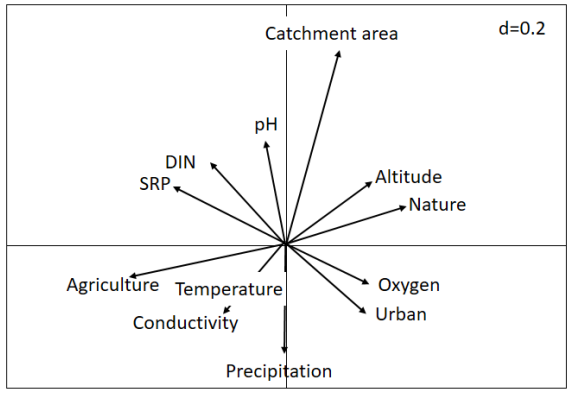
S3-Environmental characterization.

Water samples were filtered through pre-combusted glass fibre filters in the field (0.7 µm pore size; Whatman GF/F, Germany) and then transported to the laboratory under cooled conditions. In the laboratory, we

stored the water samples at 4 ºC, in darkness, until analysis (between 24 and48 hours). We analysed the concentrations of dissolved nitrite (NO_2^-) and nitrate (NO_3^-) using ionic chromatography with a conductivity detector WATERS (model 432), UV/V KONTROL detector (model 332) and the column WATER IC-PAK ANIONS (Metrohm 761 Compact IC with the column Metrosep A Supp5 - 150/4.0). We measured the ammonium concentration using the salicylate method (Reardon *et al.*, 1969) and SRP using the molybdate method (Murphy & Riley, 1962).

S3-Table 18. Principal Component Analysis correlation with the original environment characteristics. Axis 1 explained 28.67 % and axis 2 explained 21.33% of the variance.

	Axis 1	Axis 2
Temperature (ºC)	-0.29	-0.21
pH	-0.08	0.46
Conductivity (µS cm^{-1})	-0.33	-0.23
Oxygen (mg/l)	**0.74**	-0.22
SRP (ppm)	**-0.65**	0.33
DIN (ppm)	**-0.62**	0.35
Altitude (m.a.s.l)	**0.64**	0.29
Annual Precipitation (mm)	-0.01	-0.50
Order	0.09	**0.90**
Urban (%)	0.57	-0.29
Agriculture (%)	**-0.85**	-0.18
Nature (%)	**0.84**	0.18
Catchment area (km2)	0.10	**0.94**



S3-Figure 5. Loadings of original variables in the two first axis of the non-hydrological PCA (Principal Component Analysis).

S3-Analysis of water physicochemical characteristics at A and B.

To test the differences in water temperature and water concentrations of nutrients (nitrates, nitrites, ammonium and soluble reactive phosphorous; SRP) from the two streams, we performed a two-sample t-test at the 95% confidence interval level. For temperature, the differences were not significant (t-test, t_6= -0.34, p-value= 0.741). In relation to SRP and DIN (DIN = nitrite + nitrate + ammonia) concentrations, the differences were not significant in either case (t-test, t_6= -0.94, p-value= 0.367 and t_6= -1.92, p-value= 0.086, respectively).

S3-Analysis of the differences between initial aquatic hyphomycete communities at A and B.

To test differences in the initial composition of aquatic hyphomycetes colonizing leaf litter in streams A and B, we used a multivariate generalized linear model (MANYGLM model, mvabund R package). The structure of the species in the initial aquatic hyphomycetes communities associated with the leaf litter in streams A and B were significantly different (MANYGLM, p=0.001).

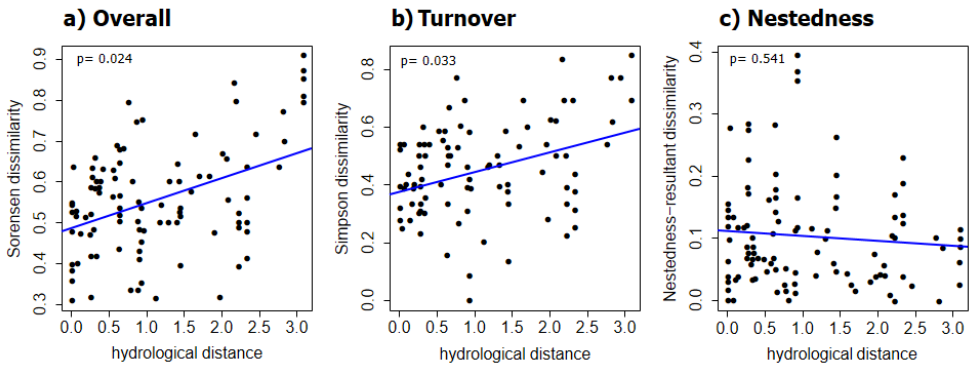
S3- Hydrologic metrics, models and results

S3-Table 19: **Pearson correlation coefficients between hydrological metrics and environmental variability (ENV).** ENV is the value of the firs axis of the PCA (see details in S1), TNF is total non-flow days, NFP is non-flow periods and NF is days of the last non-flow period.

	ENV	NF	NFP
NF	-0.24		
NFP	-0.09	0.16	
TNF	0.46	0.35	0.59

S3-Table 20: Final competing models. Grey cells indicate the variables (i.e., different flow intermittence metrics and/or ENV) included in a particular model (see **S3-Table 19** for acronyms).

	ENV	TNF	NFP	NF
Mod1	■			
Mod2		■		
Mod3			■	
Mod4				■
Mod5	■	■		
Mod6	■		■	
Mod7	■			■
Mod8	■	■	■	
Mod9	■	■		■
Mod10	■		■	■
Mod11	■	■	■	■
Mod12		■	■	
Mod13		■		■
Mod14			■	■
Mod15		■	■	■



S3-Figure 6. Beta diversity patterns.

S3-Table 21. Results of Tukey HSD for the interaction of richness and treatment on fungal biomass. A: high richness; B: low richness; P: permanent treatment; I: intermittent treatment; E: ephemeral treatment.

	Tukey HDS Richness: Treatment	
	Mean difference	p-value
B:E-A:E	-1.58	**0.000**
A:P-A:E	1.60	**0.000**
B:P-A:E	1.76	**0.000**
A:I-A:E	1.36	**0.000**
B:I-A:E	0.71	0.106
A:P-B:E	3.19	**0.000**
B:P-B:E	3.35	**0.000**
A:I-B:E	2.95	**0.000**
B:I-B:E	2.29	**0.000**
B:P-A:P	0.15	0.989
A:I-A:P	-0.23	0.937
B:I-A:P	-0.90	**0.020**
A:I-B:P	-0.39	0.649
B:I-B:P	-1.05	**0.005**
B:I-A:I	-0.66	0.143

CHAPTER 4

S4- Table 22. Consumer consumption and growth data.

	Mean (±SEM)				
Variable	t1	t2	t3	t4	t5
Total Consumption (mg leaf DM)	0.096 (±0.008)	0.076 (±0.006)	0.053 (± 0.010)	0.086 (±0.006)	0.066 (±0.007)
RCR (mg leaf DM mg larval DM $^{-1}$ day $^{-1}$)	0.171 (±0.034)	0.157 (±0.026)	0.081 (±0.019)	0.221 (±0.033)	0.125 (±0.019)
IGR (mm d^{-1})	0.019 (±0.001)	0.006 (±0.004)	0.001 (±0.003)	0.005 (±0.001)	0.007 (±0.001)
RGR mm mm^{-1} d^{-1})	0.017 (±0.001)	0.005 (±0.004)	0.001 (±0.003)	0.005 (±0.001)	0.006 (0.001)
Total lipids (%)	2.489 (±0.271)	3.536 (±0.494)	3.291 (±0.267)	4.725 (±0.745)	3.862 (±0.357)
Oxygen Consumption (mg L^{-1} mg DM^{-1} s^{-1})	0.044 (±0.016)	0.091 (±0.018)	0.035 (±0.010)	0.031 (±0.002)	0.066 (±0.004)

REFERENCES

Aspin, T. W. H., Khamis, K., Matthews, T. J., Milner, A. M., O'Callaghan, M. J., Trimmer, M., Woodward, G., & Ledger, M. E. (2019). Extreme drought pushes stream invertebrate communities over functional thresholds. *Global Change Biology*, **25(1)**, 230–244.

Belmar, O., Bruno, D., Guareschi, S., Mellado-Díaz, A., Millán, A., & Velasco, J. (2019). Functional responses of aquatic macroinvertebrates to flow regulation are shaped by natural flow intermittence in Mediterranean streams. *Freshwater Biology*, **64(5)**, 1064–1077.

Bruno, D., Belmar, O., Maire, A., Morel, A., Dumont, B., & Datry, T. (2019). Structural and functional responses of invertebrate communities to climate change and flow regulation in alpine catchments. *Global Change Biology*, **25**, 1612-1628.

Chessman, B. C. (2015). Relationships between lotic macroinvertebrate traits and responses to extreme drought. *Freshwater Biology*, **60(1)**, 50–63.

Díaz, A. M., Alonso, M. L. S., & Gutiérrez, M. R. V. A. (2008). Biological traits of stream macroinvertebrates from a semi-arid catchment: Patterns along complex environmental gradients. *Freshwater Biology*, **53(1)**, 1–21.

Laliberte, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, **91(1)**, 299–305.

Laliberté, E., Wells, J. A., Declerck, F., Metcalfe, D. J., Catterall, C. P., Queiroz, C., Aubin, I., Bonser, S. P., Ding, Y., Fraterrigo, J. M., McNamara, S., Morgan, J. W., Merlos, D. S., Vesk, P. A., & Mayfield, M. M. (2010). Land-use intensification reduces functional redundancy and response diversity in plant communities. *Ecology Letters*, **13(1)**, 76–86.

Maire, E., Grenouillet, G., Brosse, S., & Villéger, S. (2015). How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Global Ecology and Biogeography*, **24(6)**, 728–740.

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36.

Pavoine, S., Vallet, J., Dufour, A.-B., Gachet, S., & Daniel, H. (2009). On the challenge of treating various types of variables: application for improving the measurement of functional diversity. *Oikos,* **118(3)**, 391–402.

Reardon, J., Company, C.M. & Valley, S. (1969) Salicylate method for the quantitative determination of amonia nitrogen. *United States Patent Office*, **11**, 3–6.

ORIGINAL PUBLICATIONS

Nuestras vidas son los ríos

que van a dar en la mar,

que es el morir;

allí van los señoríos

derechos a se acabar

y consumir;

allí los ríos caudales,

allí los otros medianos

y más chicos,

y llegados, son iguales

los que viven por sus manos

y los ricos.

-Jorge Manrrique -

PLOS ONE

RESEARCH ARTICLE

Quality and quantity of leaf litter: Both are important for feeding preferences and growth of an aquatic shredder

Rebeca Arias-Real[1]*, Margarita Menéndez[1], Meritxell Abril[2], Francesc Oliva[3], Isabel Muñoz[1]

1 Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain, **2** BETA Technological Centre, University of Vic-Central University of Catalonia, Vic, Spain, **3** Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain

* rebeca.arias.real@ub.edu

OPEN ACCESS

Citation: 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(12): e0208272. https://doi.org/10.1371/journal.pone.0208272

Editor: Luiz Ubiratan Hepp, Universidade Regional Integrada do Alto Uruguai e das Missoes, BRAZIL

Received: August 13, 2018

Accepted: November 14, 2018

Published: December 12, 2018

Data Availability Statement: In the supporting information (S1 Table).

Funding: This work was funded by the Ministry of Economy, Industry and Competitiveness of Spain via the projects CGL2014-58760-C3-1-R and CGL2017-88640-C2-2-R. RAR held a predoctoral grant (BES-2015-073961, FPI) from the Ministry of Economy, Industry and Competitiveness of Spain. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

The study of leaf litter as a resource for shredders has emerged as a key topic in trophic links in ecology. However, thus far, most studies have emphasized the leaf quality as one of the main determinants of shredder behaviour and growth without simultaneously considering the leaf quantity availability. Nevertheless, the combined effects of leaf quantity and quality on shredder behaviour and growth is particularly crucial to further understand how ecosystem functioning may respond to the increasing flow intermittency due to climate change. In this study, we explore how changes in the leaf litter quality and quantity influence the feeding preferences and growth of an invertebrate shredder (*Potamophylax latipennis*). To do so, we used black poplar leaves conditioned in two streams with different flow regimens as a food resource. Afterwards, using a microcosm approach, we offered leaf discs that varied in terms of leaf quantity and quality to *P. latipennis*. Our results showed that flow intermittency had a negative effect on the quality of the food resource, and a lower quality had a negative effect on the consumption and growth rates of *P. latipennis*. Furthermore, we found that *P. latipennis* fed selectively on higher quality leaves even though the availability (quantity) of this resource was lower. In the context of climate change, with higher aridity/drier conditions/scenarios, our findings suggest that a decrease in the availability (quantity) of high-quality resources could potentially threaten links in global fluvial food webs and thus threaten ecosystem functioning.

Introduction

Rivers that naturally and periodically cease to flow in time and/or space are termed intermittent rivers (IRs) and are recognized as the most common fluvial ecosystem around the world [1]. The seasonal flow variability in IRs is the most important factor that determines their functioning; for instance, this variability determines the nutrient dynamics or hydrological connectivity essential for community dispersion [2].

Competing interests: The authors have declared that no competing interests exist.

As a result of the increasing aridification caused by climate change, many streams are expected to become IRs, experiencing greater variability in their water flow, which may eventually lead to complete flow disruption [3]. Despite the importance and increasing abundance of IRs, the effects of changes in water flow on biodiversity and ecosystem functioning are still largely unknown [4].

Organic matter decomposition is a key ecosystem process that influences the cycling of nutrients and energy flow to higher trophic levels [5,6]. Microbial decomposers (fungal and bacterial communities) and invertebrate shredders are the biological drivers of organic matter decomposition. Among the microbial decomposers, aquatic hyphomycetes (fungal community) are considered the first colonizers and main drivers of microbial decomposition in its first stages [7], which constitutes an important trophic link between leaf litter and shredders [8]. Aquatic hyphomycetes improve the palatability and nutritional quality (increasing the proteins, lipids and carbohydrates due to the characteristics of the fungi themselves) of leaf litter by transforming recalcitrant polymers into more labile molecules via their enzyme capabilities [9,10] and increasing the nitrogen and phosphorus concentrations of leaf litter via the accumulation of fungal mycelia [11,12]. This leaf transformation is crucial for shredders, which need a critical amount and balance of inorganic and organic elements for growth, reproduction and maintenance [9]. Moreover, the shredders cannot synthesize some essential components (e.g. essential fatty acids) and must therefore acquire them from their diet [13]. Consequently, shredders tend to consume the most optimal resource, which is the most energetic and nutrient-rich food available.

Generally, flow reduction affects the communities of aquatic hyphomycetes, which are particularly vulnerable to desiccation stress, especially those that are not adapted to flow reduction [14]. Some studies have shown that during flow reduction, the communities of aquatic hyphomycetes can experience a shift in fungal richness and composition and alterations in their enzymatic activity, such as summer drying conditions inhibiting lignocellulolytic enzyme activities [10,15,16]. These changes in the communities of aquatic hyphomycetes coupled to changes in the abiotic conditions of streams under flow reduction (e.g., decreasing the dissolved oxygen content and increasing the water temperature and conductivity) are expected to affect organic matter decomposition and the feeding links to higher trophic levels [17,18].

In addition, flow reduction also affects the riparian vegetation, causing early leaf abscission [19]. This may lead to temporal and spatial changes in this basal resource for aquatic hyphomycetes and shredders. Despite its potential implications for the organic matter cycle, the effects of decreasing the availability of high-quality resources, such as leaf litter, on consumers is still poorly studied.

To date, studies have focused on leaf quality to explain invertebrate shredder behaviour and growth [20–22]. For example, Frost et al. [23,24] have shown that shredders compensated for nutrient limitations by increasing feeding rates or selectively feeding on resources with more nutritious properties. Nevertheless, little is known regarding the effect of combined changes in leaf quantity and quality on the feeding preferences of invertebrate shredders and their growth. This knowledge is particularly crucial for understanding how biodiversity (e.g., aquatic hyphomycetes community) and ecosystem functioning (e.g., organic matter decomposition and its implications on stream food webs [22]) may respond to ongoing effects of climate change.

In line with this information, we address this knowledge gap herein by exploring how changes in both the leaf litter quality and quantity affect or determine the feeding preferences and growth of an invertebrate shredder using field and microcosm approaches. To do so, we first assessed the influence of flow intermittency on the leaf litter quality (thought fungal biomass, C:N ratios and total lipid content) and the composition of the associated community of aquatic hyphomycetes. We expected that under flowing conditions (a permanent stream) the

leaf litter would be of better quality (lower C:N ratios and higher fungal biomass and lipid content) than that from an IRs. Second, we explored the joint effects of leaf litter (food resource) quality and quantity on the consumption rates and growth of a shredder using microcosms. We expected that better quality food resources and availability would be correlated with higher consumption rates and growth of the shredder. Finally, we quantified the feeding preferences of the shredder, expecting that the quality rather the quantity of the resource would be more important. Therefore, even if the shredder has a larger quantity of poor-quality resources, they will actively select the best quality food.

Materials and methods

No specific permissions were required for my locations/activities. Our study did not involve endangered or protected species.

Leaf litter and fungal assemblages

We used black poplar leaves (*Populus nigra* L.*)* conditioned in two different streams as a food resource. We selected one permanent stream (Arbucies in the Tordera Basin, N 41˚ 823133 E 2˚ 452826; hereafter termed "A") and one intermittent stream (Llobina in the Besos Basin, N 41˚ 46.011 E 2˚ 16.104; hereafter termed "B") in Catalonia, Spain. Both streams are third-order streams, meaning that they have different flow regimes but similar water physicochemical characteristics [25]. Furthermore, both streams have the same geology (siliceous bedrock) and poplar (*Populus nigra* L.), alder (Alnus glutinosa (L.) Gaertner) and evergreen oak (Quercus ilex L.O) are the dominant riparian vegetation. In addition, previous studies have indicated differences in the biodiversity of aquatic hyphomycetes between these streams [26], with lower diversity in the Llobina stream (B).

To characterize the stream hydrology, in February 2016, we placed Leveloggers (Solinst Levelogger Edge, full-scale reading precision of 0.05%) on the streambed for water level and temperature recordings as a proxy to measure the presence or absence of flow. The Leveloggers were recorded at hourly intervals for one year (from February 2016 until January 2017). The recorded data were corrected with barometric pressure variations using data from Barologgers (Solinst Barologger, full-scale reading precision of 0.05%). We installed the Barologgers at each site in the riparian area to measure the atmospheric pressure changes. In addition, throughout this year, we collected samples two times (in October 2016 and January 2017) to analyse the water concentrations of nutrients (nitrate, nitrite, ammonium and soluble reactive phosphorous (SRP)) from both streams.

Black poplar leaves were collected freshly abscised in autumn 2016 from the riparian area of A stream and dried at room temperature until needed. The dried leaf sets were inserted into mesh bags (0.5 mm mesh size and with approximately 10–12 leaves) and transferred to the streams (23 mesh bags in each stream) along a 100 m reach.

As previous studies demonstrated that the conditioning process follows a unimodal pattern in which the palatability of leaves increases to a maximum within 2 to 6 weeks and then declines, we removed the leaf mesh bags from each stream after 20 days of conditioning [27,28]. Afterwards, the mesh bags were transferred to the laboratory and 945 leaf discs of 16 mm diameter were obtained with a cork borer, avoiding veins. We repeated this process twice (with a week in between) to supply similar food conditions to the shredder (see below).

Leaf litter quality after 14 days of conditioning

We used a set of leaf discs from each stream to characterize the initial communities of aquatic hyphomycetes. To induce conidial release from the hyphomycetes, five leaf discs with six

replicates per stream were placed into 10 Erlenmeyer flasks with 60 ml of dechlorinated water at 14˚C under 12 h light: 12 h dark conditions for 48 h. We aerated the Erlenmeyer flasks from the bottom by a continuous airflow to create turbulence that kept the leaf discs in continuous motion [29]. To determine the initial communities of aquatic hyphomycetes in the leaf discs, we filtered the suspensions of spores through 5-μm-pore size membrane filters (Cellulose Nitrate Membrane Filters, Whatman) and stained the filters with one drop of Trypan Blue solution containing lactic acid. For all the samples, we filtered the same volume (5 ml) of the suspensions of spores. Then, we used fields for identify and count the spores. First, we scanned the surface of the filter under the light microscope (400x), then we counted and identified all conidia and if they were very numerous, we counted the conidia in 20–30 randomly chosen microscope fields. We expressed the result as number of conidia per mL. Furthermore, based on these results we created a presence or absence conidia matrix [30].

The ergosterol concentration in the leaf discs was determined as a proxy for fungal biomass [31]. Five frozen leaf discs per five replicates per stream were lyophilized and weighed to determine the dry mass. We performed the lipid extraction and saponification using 0.14 M KOH methanol (8 g L^{-1}) at 80˚C for 30 min in a shaking water bath. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak, Vac RC, 500 mg, tC18 cartridges, Waters Corp., Milford, MA, USA), and ergosterol was eluted using isopropanol. We used high pressure liquid chromatography (HPLC) to detect and quantify the ergosterol by measuring the absorbance at 282 nm. We used a Jasco HPLC system (USA) equipped with a Gemini-NX 5 μm C18 250 × 4.6 mm column (Phenomenex, UK). The mobile phase was 100% methanol, and the flow rate was set to 1.2 ml min^{-1}. Finally, we converted the ergosterol into fungal biomass using a conversion factor of 5.5 mg of ergosterol per gram of fungal mycelium [32]. We expressed the results in mg of fungal biomass per gram of dry mass leaf litter.

To determine the total lipid content in the leaf, we lyophilized five frozen leaf discs per five replicates per stream and homogenized them with an ultrasonic homogenizer (200 W, 24 kHz; Hielscher Ultrasonics GmbH, Teltow, Germany). We performed the lipid extraction with a monophasic solution of chloroform and methanol (2:1, v/v). Then, using a biphasic solution (chloroform and distilled water), we separated the phases, and after one night at 50˚C, we analysed the total lipid content using the colorimetric sulpho-phospho-vanillin method [33]. We expressed the results as the percentage of total lipid.

Finally, another set of five leaf discs per five replicates from each stream was dried and ground into a fine powder to analyse the nitrogen (N) and carbon (C) concentrations using a Thermo Element Analyzer 1108 (Thermo Scientific, Milan, Italy). We expressed the results in terms of C:N molar ratios.

Shredder

We collected individuals of *Potamophylax latipennis* (Trichoptera: F. Limnephilidae, Curtis, 1834) in stream A in February 2017 and transported them to the laboratory in plastic containers containing stream water and sand. To acclimatize the shredders to the laboratory conditions, they were maintained in declorinated water with food (leaves from the river) provided ad libitum for three days before starting the experiment. The temperature was maintained at 14˚C. On the third day, the shredders were starved, which allowed evacuation of their gut contents. Once the shredders were acclimatized, we sorted 86 shredders with similar size. To calculate the average initial larval weight, average initial head width and the average initial lipid content, we separated thirty-six individuals we measured and frozen them at -80˚C, lyophilized, and weighed (initial head width = 1.79 ± 0.03 mm; initial larvae dry mass = 0.031 ± 0.07 mg, n = 36).

Microcosm setup

We allocated the shredders individually into fifty glass microcosms (8.5 cm diameter and 8.3 cm height) with 60 ml of dechlorinated water. We added 12 leaf discs in each microcosm according to five different treatments: Treatment 1 (t1), ten microcosms with leaf discs from only stream A; treatment 2 (t2), ten microcosms with leaf discs from stream B; treatment 3 (t3), ten microcosms with equal proportions of leaf discs from streams A and B; treatment 4 (t4), ten microcosms with 75% of the leaf discs from stream A and 25% of the leaf discs from stream B; treatment 5 (t5), ten microcosms with 25% of the leaf discs from stream A and 75% of the leaf discs from stream B. In mixture treatments (from t3 to t5), discs from each type of leaves (A or B) were marked with colour pins. In addition, twenty microcosms containing leaf discs (ten with leaf discs from only stream A and ten with leaf discs from stream B) were maintained without shredders to serve as the controls for the loss of leaf not attributable to consumption (Fig 1).

We provided each microcosm with ash sand from the same riverbed (previously burnt at 450˚C for 4 h) to allow larvae to build their cases. We aerated the microcosms by a continuous airflow under 12 h light: 12 h dark photoperiod conditions at 14˚C in incubator rooms. The experiment lasted 2 weeks. Every three days during the experiment, we controlled the NH_4 concentration of each microcosm (Tetra Test NH_3/NH_4, Tetra GmbH, Germany) and the water level. If the water level was below 60 ml, we added dechlorinated water until that level was reached. At the end of the first week, we replaced the water and leaf discs from those colonized one week later in the streams. All leaf discs were kept frozen at -80˚C until analysis.

Shredder consumption and growth

We determined the shredder consumption (C, mg) at the end of the experiment in each treatment as follows:

$$C = \sum_{k=1}^{2}(Li - Lf) \quad (1)$$

where k is the number of weeks of the experiment, and Li and Lf are the initial and final dry masses (hereafter DM, mg) of leaf discs for each week of the experiment, respectively, corrected by the DM leaf loss in the control microcosms without shredders of the respective treatments.

We calculated the relative consumption rate per larvae (RCR, mg leaf DM mg^{-1} larval DM day^{-1}) as follows:

$$RCR = \frac{C/T}{w} \quad (2)$$

where T is the time for the entire feeding period (14 days) and w is the average of the larval DM (mg) at the beginning and end of the experiment.

We calculated the instantaneous growth rate (IGR, mm d^{-1}) and the relative growth rate (RGR, mm mm^{-1} d^{-1}) using the head width (HW) of the larvae [12] as follows:

$$IGR = \frac{\ln(HWf) - \ln(HWi)}{T} = \frac{\ln(\frac{HWf}{HWi})}{T} \quad (3)$$

and

$$RGR = \frac{HWf - HWi}{(HWf * T)} \quad (4)$$

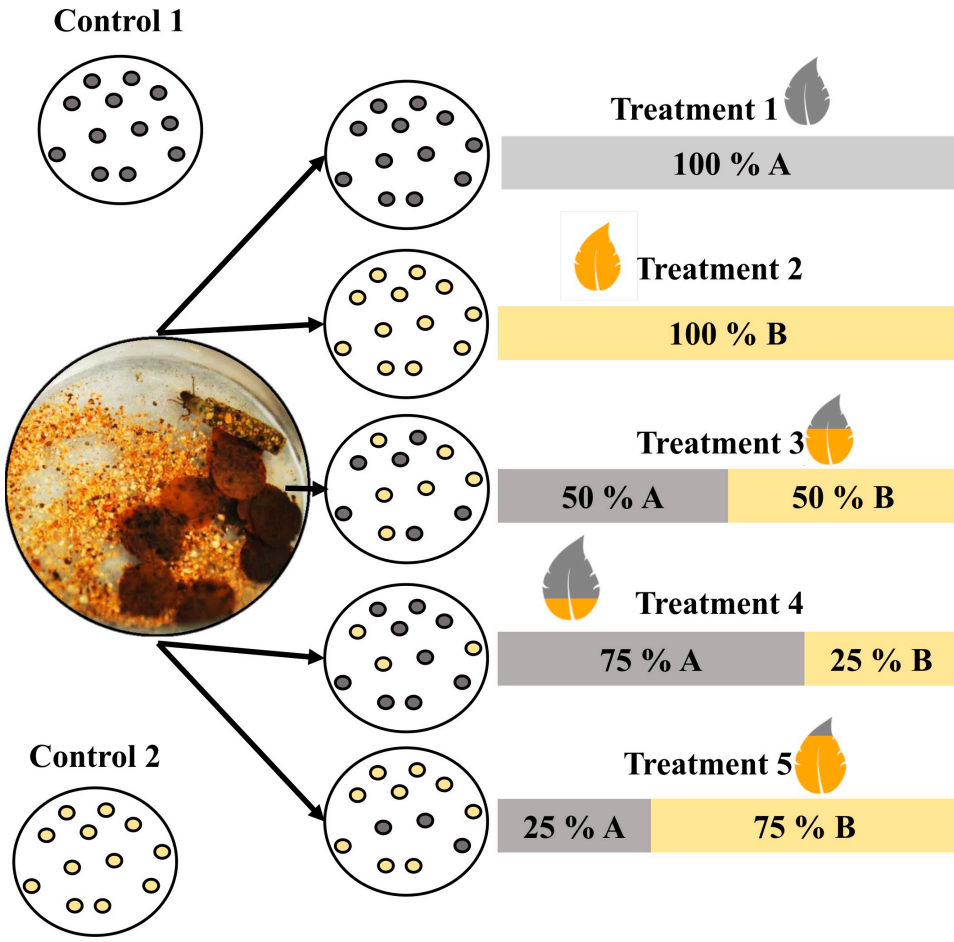


Fig 1. Experimental design. A and B refer to the leaf discs from the permanent and intermittent streams, respectively. Each microcosm contained individual shredders and 12 leaf discs. Ten microcosms were used per treatment.

https://doi.org/10.1371/journal.pone.0208272.g001

where *HWf* and *HWi* are the final and initial head width (mm), respectively and *T* is the time for the entire feeding period (14 days).

We measured the metabolism via the oxygen consumption for nearly 10 min and corrected the measurement by the individual dry weight (mg O_2 L^{-1} g DM^{-1} min^{-1}). Measurements were made with an optical oxygen microsensor adapted to a 20 ml glass vial (Fibox 4 PreSens, Regensburg, Germany) filled with the oxygen-saturated water in which the shredder had been introduced. The oxygen concentration was recorded every 5 seconds for 10 min [34].

Finally, as a proxy for their body condition, we analysed the total lipid content of each shredder, expressed as a percentage of invertebrate DM. At the end of the experiment, each individual was frozen separately at -80˚C. The protocol was similar to that for the leaves. We quantified the lipid content by spectrophotometry after digestion with H_2SO_4 (100˚C) and comparison against a cholesterol standard [9].

Data analysis

To characterize the stream hydrology in the intermittent stream, we used the daily variation of the streambed temperature corrected for the barometric pressure and air temperature. This daily variation was determined as the difference between the maximum and minimum temperature per day and the daily higher rate of change per hour. We performed a fifth-order moving average to smooth daily differences. To test the differences in water temperature, water concentrations of nutrients (nitrate, nitrite, ammonium and soluble reactive phosphorous (SRP)) from both streams, we performed a two samples t-test at the 95% confidence interval level.

To analyse the effects of flow regime on the leaf litter quality (chemical composition of leaf litter) and richness of aquatic hyphomycetes between streams A and B, we performed two sample t-tests at the 95% confidence interval level. To test differences between the composition of the conidia produced by the aquatic hyphomycetes colonizing the leaf litter from streams A and B, we used a multivariate generalized linear model (MANYGLM model, mvabund R package) due to it is a flexible and powerful framework for analysing abundance data and show a better power properties than distance-based methods [35]. Indicator taxa were defined for each stream class (A and B) using the indicator species analysis (IndVal) of Dufrene and Legendre [36]. This analysis generates an indicator value index (IV) for each species (based on the presence or absence of a spore matrix) and stream class. The indicator calculation is based on the specificity (maximum when the species occurs in only one stream) and fidelity (maximum when the species is present in both streams). To perform the tests, we used the packages vegan, mvabund, labdsv and ade4 in R.

To evaluate the differences between treatments in the endpoints measured in the larvae, we first checked the outliers of the data, the variable distributions (skewness) and the assumption of normality (Bartlett and Shapiro test). For variables that did not fulfil the assumptions of normality, we transformed the original data using a square root transformation for RCR and total lipid and a $\log_{10}$ (x+1) transformation for RGR and IGR. We performed one-way ANOVA using the treatment as the fixed effects factor with the car and sandwich packages in R. We validated the model visually by assessing the distribution of residuals for normality and homoscedasticity [37]. When the null hypothesis was rejected, we performed post hoc Tukey pairwise comparisons using the multcomp package in R.

Finally, to analyse the effect of leaf quantity on feeding preferences of *P. latipennis*, we performed a one sample t-test for the three-mixture treatment to compare the observed consumption of A leaf discs to the total consumption and the expected value, considering the last one as the real proportion of A leaf discs at each treatment (50%, 75% and 25%).

All the statistical analyses were performed using the R statistical software version 3.4.1 [38], with the significance level set at $p < 0.05$ for all tests. The datasets used in this study are available in S1 Table.

Results

The intermittent stream presented a summer drought with 46 dry days and an average temperature of 10.06˚C (± 1.99), whereas the permanent stream presented an average temperature of

10.70 (± 1.90) (differences were not significant, t-test, t_6 = -0.34, p-value = 0.741). The intermittent stream SRP was 0.008 ppm (± 0.001), and the permanent stream SRP was 0.013 ppm (± 0.002) (t-test, t_6 = -0.94, p-value = 0.367). The total dissolved inorganic nitrogen (DIN = nitrite + nitrate + ammonia) concentration was 0.24 (± 0.07) in the intermittent stream and 0.41 ppm (± 0.13) in the permanent stream (t-test, t_6 = -1.92, p-value = 0.086).

Effects of flow regime on the leaf litter quality

The flow regimen significantly affected the quality of the leaf litter after 20 days of conditioning in streams A and B. The quality differed on lipid content, fungal biomass and aquatic hyphomycetes richness (Table 1) being higher for the leaf litter conditioning in stream A.

The structure of the species in the initial aquatic hyphomycetes communities associated with the leaf litter in streams A and B were significantly different (MANYGLM, p = 0.001). IndVal analysis revealed that the species *Heliscus submersus*, *Alatospora acuminta*, *Tetrachaetun elegans* and *Lemonniera aquatica* were significantly different, and the species *Fusarium* sp. and *Articulospora tetracladia* were marginally significantly different between the streams. Moreover, *Cilindrocarpon* sp., *Alatospora acuminata*, *Tetracladium setigerum*, *Tetracladium marchalianum*, *Anguillospora longuissima*, *Tricladium chaetocladium*, *Lemonniera aquatica* and *Clavariopsis aquatica* appeared in only stream A, whereas *Dendrospora* sp. appeared in only stream B (Table 2). However, MANYGLM analysis also revealed that the dominant species in both streams *Flagellospora curvula*.

Effects of leaf litter quality and quantity on *P. latipennis*

Differences in the leaf litter quality between streams A and B significantly affected the consumption and growth of *P. latipennis* (Table 3).

Total consumption was significantly higher in treatments with a higher proportion of A leaf discs, i.e., t1 and t4 treatments with 100% and 75% of A leaf discs, respectively. Post hoc comparisons also showed significant differences (Tukey HSD, $p<0.05$) between treatments t1 and t4 and treatments with a lower proportion of A leaf discs, i.e., t3 and t5 treatments, with 50% and 25% of A leaf discs, respectively (Fig 2A). The RCR was also significantly lower in treatment t3 (50% of A leaf discs) than in the t1 and t4 treatments (Tukey HSD, $p<0.05$; Fig 2B).

The IGR based on head larvae width was 0.019 mm ± 0.001 per day for t1 (Fig 2C), whereas for the rest of the treatments, the head width showed lower IGRs (mean ± SEM, t2 = 0.005 ± 0.004, t3 = -0.000 ± 0.003, t4 = 0.005 ± 0.001 and t5 = 0.007 ± 0.001), and significant differences were found between treatments t1 and the others (Tukey HSD, $p<0.05$). The RGR (Fig 2D) were significantly different between t1 and t2 (Tukey HSD $p<0.05$). Therefore, when the larvae were fed with leaf discs of higher quality (t1, 100% A leaf discs), their relative growth was higher (mean ± SEM, t1 = 0.017 ± 0.001, t2 = 0.004 ± 0.001), and even in the RCR, no significant differences were observed (Fig 2B). Moreover, there were also significant

Table 1. Means ± SEM of the initial chemical leaf litter composition (n = 5) and aquatic hyphomycetes richness (n = 6) and t-tests results for the quality of the leaves in both treatments (A and B).

	Mean (± SEM)		Statistics	
Variable	Permanent (A)	Intermittent (B)	t_8	p-value
Total lipid (%)	4.13 (± 0.07)	3.36 (± 0.02)	9.45	**<0.001**
C:N	63.5 (± 2.9)	51.2 (± 1.5)	2.42	0.051
Fungal Biomass (mg FB/g DM)	33.71 (± 4.02)	17.68 (± 5.09)	4.30	**0.004**
Aquatic hyphomycetes richness	12 (± 0.4)	5 (± 0.6)	8.87	**<0.001**

https://doi.org/10.1371/journal.pone.0208272.t001

Table 2. Results of the indicator species analysis (IndVal), maximum IV significance (IV is the individual value), associated stream class for each species and the frequency of appearance (A and B, permanent and intermittent streams).

Species	Class	IV	p-value	A	B
Flagellospora curvula	1	0.500	1	100	100
Heliscus lugdunensis	1	0.600	0.461	100	66.7
Heliscus submersus	1	0.750	**0.012**	100	16.6
Tetrachaetum elegans	1	0.857	**0.024**	100	16.67
Fusarium sp.	2	0.694	0.062	16.6	83.3
Cylindrocarpon sp.	1	0.667	0.075	66.6	0
Alatospora acuminata	1	1.000	**0.007**	100	0
Articulospora tetracladia	1	0.694	0.074	83.3	16.6
Lemonniera pseudofloscula	1	0.521	0.567	83.3	50
Lemonniera aquatica	1	0.667	**0.049**	66.6	0
Dendrospora sp.	2	0.167	1	0	16.6
Tetracladium setigerum	1	0.167	1	16.6	0
Tetracladium marchalianum	1	0.500	0.205	50	0
Anguillospora longissima	1	0.333	0.453	33.3	0
Tricladium chaetocladium	1	0.167	1	16.6	0
Clavariopsis aquatica	1	0.333	0.446	33.3	0

https://doi.org/10.1371/journal.pone.0208272.t002

differences between t1 and t3, and surprisingly, between t1 and t4 (Tukey HSD $p<0.001$ and $p = 0.010$, respectively). The t4 treatment showed higher consumption but lower growth. Larvae of this treatment showed a greater proportion of lipids than t1 (Tukey HSD, $p = 0.019$) (Fig 2E).

The metabolism of the shredder larvae was significantly higher in t2 (100% B leaf discs) than in the other treatments (Tukey HSD, $p<0.005$) except for t5, in which 75% of the leaves were from B (mean ± SEM, t1 = 0.04 ± 0.01; t2 = 0.09 ± 0.01; t3 = 0.03 ± 0.01; t4 = 0.03 ± 0.02 and t5 = 0.06 ± 0.01) (Fig 2F). No mortality was observed during the experiment.

When leaf discs from stream A and B were offered simultaneously in different quantities to the shredders (mixture treatments: t3, t4 and t5), the shredders showed a tendency to select leaf discs from A (t-test, $t_{14} = 2.184$, $p = 0.047$).

The percentages of the quantity of A leaf discs consumed regarding the consumption expected at each treatment according to the quantity of each type of leaf disc were mostly greater than the expected consumption at each treatment (Fig 3). In t3, the larvae consumed 65% of A leaf discs, whereas the expected amount was 50% (15% more than expected), while in t4, larvae consumed 77% and 75% was expected (2% more than expected), and finally, the t5 larvae consumed almost

Table 3. Results of one-way ANOVA (factor treatment) of the effects of leaf quality on consumer consumption and growth.

Variable	Treatment		
	df	F	P
Total Consumption (mg leaf DM)	4	4.481	**0.004**
RCR (mg leaf DM mg larval DM $^{-1}$ day $^{-1}$)	4	4.317	**0.005**
IGR (mm d^{-1})	4	5.622	**0.001**
RGR (mm mm^{-1} d^{-1})	4	5.074	**0.002**
Total Lipids (%)	4	2.877	**0.035**
Oxygen Consumption (mg L^{-1} $mgDM^{-1}$ s^{-1})	4	3.926	**0.041**

N = 50 (10 replicates per treatment) except for oxygen consumption, where n = 15 (three replicates per treatment).

https://doi.org/10.1371/journal.pone.0208272.t003

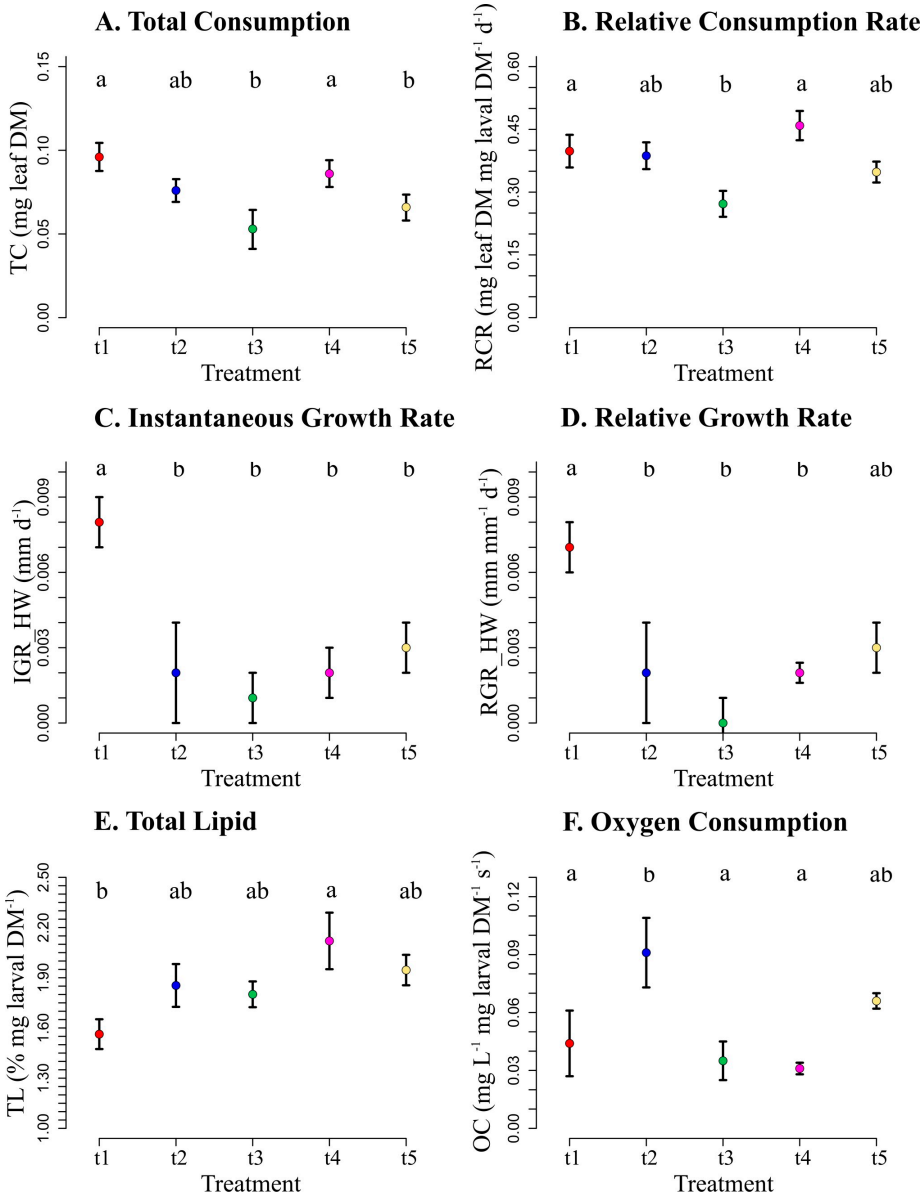

A. Total Consumption
TC (mg leaf DM)
0.00
0.05
0.10
0.15
a
ab
b
a
b
t1
t2
t3
t4
t5
Treatment
B. Relative Consumption Rate
RCR (mg leaf DM mg laval DM-1 d-1)
0.00
0.15
0.30
0.45
0.60
a
ab
b
a
ab
t1
t2
t3
t4
t5
Treatment
C. Instantaneous Growth Rate
IGR_HW (mm d-1)
0.000
0.003
0.006
0.009
a
b
b
b
b
t1
t2
t3
t4
t5
Treatment
D. Relative Growth Rate
RGR_HW (mm mm-1 d-1)
0.000
0.003
0.006
0.009
a
b
b
b
ab
t1
t2
t3
t4
t5
Treatment
E. Total Lipid
TL (% mg larval DM-1)
1.00
1.30
1.60
1.90
2.20
2.50
b
ab
ab
a
ab
t1
t2
t3
t4
t5
Treatment
F. Oxygen Consumption
OC (mg L-1 mg larval DM-1 s-1)
0.00
0.03
0.06
0.09
0.12
a
b
a
a
ab
t1
t2
t3
t4
t5
Treatment

Fig 2. Shredder consumption and growth. Total consumption (A), relative consumption rate (RCR) (B); instantaneous growth rate of the head width (IGR) (C); relative growth rate of the head width (RGR) (D); total lipid content (E) and, oxygen consumption (F) of *P. latipennis*, where t1 = 100% A, t2 = 100% B, t3 = 50% A and 50% B, t4 = 75% A and 25% B and t5 = 25% A and 75% B. The different letters indicate significant differences (Tukey HSD post hoc test, $p < 0.05$) among treatments for each variable.

https://doi.org/10.1371/journal.pone.0208272.g002

all the A leaf discs (27% more than expected). When A leaf discs were offered below 50% (treatment 5), the shredder fed selectively A leaf discs (t-test, $t_5 = 2.5379$, $p = 0.042$).

Discussion

Our results showed that flow intermittency reduces the quality of leaf litter in terms of fungal richness and biomass and lipid content (*Objective 1)*. In addition, these changes in food quality

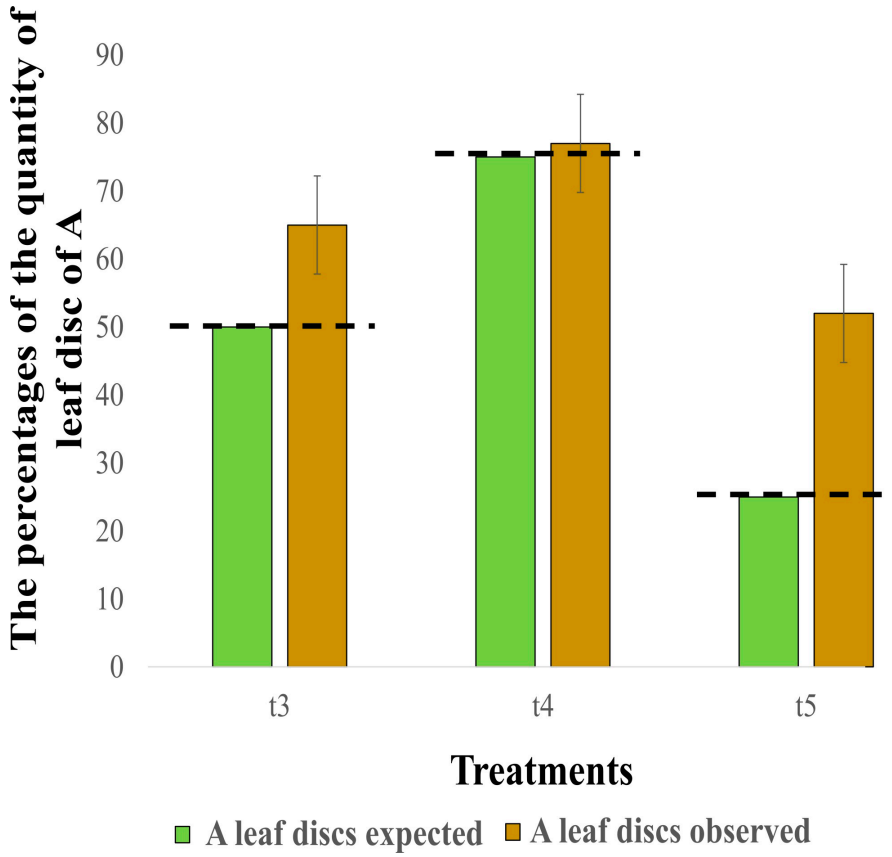


Fig 3. The observed consumption (black bars) of A leaf discs compared with the expected consumption (grey bars). The expected A leaf discs as the initial proportion of A discs at each treatment: t3 = 50%, t4 = 75% and t5 = 25%). N = 5 (t3, t4) and N = 4 (t5).

https://doi.org/10.1371/journal.pone.0208272.g003

influenced the consumption rates (i.e., the leaf litter most consumed were those conditioned under permanent flow) and growth of the shredder (*Objective 2*). Finally, *P. latipennis* fed selectively on higher quality leaves; although its availability (quantity) was lower (*Objective 3*).

Effects of flow regime on leaf litter quality

This study pointed out that flow regime influenced the leaf litter quality by means of changes in fungal colonization. Permanent flow allowed the continuous colonization of leaf litter, resulting in higher fungal richness, biomass and lipid content than leaf litter conditioned under intermittent flow conditions.

According to Suberkropp et al. [39], hyphomycete richness affects the palatability of the leaf resources, as a higher leaf litter quality is associated with fungal composition and richness. Several studies reported that shredders preferentially fed on well-conditioned leaf litter [12,15], probably related to the characteristics of the fungi themselves (high nutritional value, [40]) and to the chemical modifications of leaf litter by fungi [10]. Different fungal species have different degradative capabilities that make leaf litter more palatable. Previous studies [39,40] demonstrated that *A. acuminata*, *C. aquatica*, *F. curvula*, *L. aquatica and T. marchalianum* had the capacity to produce enzymes that degraded polygalacturonic acid, xylan and carboxymethyl cellulose. Our results showed that the abundances of most of these species (except *F. curvula*) were higher on leaf litter conditioned under permanent than intermittent flow condition. Another species, *T. elegans*, has similar enzyme capabilities [41], and this species was more abundant in the permanent stream. Furthermore, *H. lugdunensis* has been reported as a fungal colonizer preferred by shredders [42], and this specie appears in a higher abundance in leaf litter colonized under continuous flow. All of these data reinforce the idea that permanent flow conditions promoted a higher fungal richness on leaf litter, and therefore, a higher litter quality than under intermittent flow conditions. To further explore this idea, we suggest that additional studies should be conducted using molecular analysis to evaluate the roles of other low-abundance fungal species that might be relevant in terms of leaf palatability. Such studies will provide knowledge on specific fungal traits and enzymatic activities.

In addition to fungal richness, our results also pointed out that higher leaf-associated fungal biomass occurred under permanent than intermittent flow conditions, which is consistent with previous studies [43–45]. While flow disruption constrained and retarded fungal growth and colonization, permanent flow stimulated the sporulation process and supplied a continuous source of fungal spores to leaf litter [10]. A higher fungal biomass is related to a higher litter quality and palatability, attributable to an enrichment of N due to the uptake and immobilization of this element from the water column by fungal communities [46].

The lower fungal biomass found under intermittent flow conditions also influenced the lipid content, as demonstrated in other studies (e.g., [9,19,45]). Flow intermittency determines a reduction in the total and essential fatty acids in leaf litter [19,47], which influences its quality. Müller-Navarra et al. [48] found that the contents of lipids, such as fatty acids, including polyunsaturated fatty acids, is essential and can limit consumer growth, reproduction, neural development and trophic transfer efficiency. In accordance, the higher total lipid concentration found in leaf litter colonized in the permanent stream led to a better quality [49,50] for consumers.

Finally, molar C:N ratios are considered an important indicator of the nutritional value of food resources due to the positive correlation between nitrogen content and shredder preferences [51]. Unfortunately, in our results, we did not find significant differences in the C:N ratios for leaf litters conditioned in the two streams.

Effects of the leaf quality and quantity on consumer consumption and growth

Several studies have shown the importance of nutritional quality of the leaf litter resource for consumer feeding preferences and growth. Gonçalves et al. [5,15] highlighted the importance of fungal composition and richness on shredder feeding rates, and Arsuffi & Suberkropp [52] showed the importance of lipids and proteins for stimulating shredder consumption, as shredders cannot synthesize these components and must therefore acquire them from their diet [13,53].

The results of our experiment showed that despite the differences in leaf quality, the total consumption and RCR between the leaf litters conditioned in both streams were not significantly different. The similar consumption rates observed in our study between the t1 and t2 treatments (100% leaf discs from A and B, respectively) could be related to the fungal composition of the leaf litter in both streams, among other factors. As indicated previously, two fungal species reported as being highly palatable to shredders (*F. curvula and H. lugdunensis)* were abundant in the leaf litters conditioned in both streams [42,43,52,54]. However, when we simultaneously offered leaf discs from both streams (treatments t3, t4 and t5), shredders consumed less when A leaf discs were in a lower proportion (t3, t5; 50% and 25%, respectively) in relation with t1 and t 4 (100% and 75%, respectively). There was a preferential selection of A leaf discs in all mixture treatments, as demonstrated by the feeding preference results (Fig 3). The higher fungal biomass and lipid content of the A leaf discs together with their fungal composition stimulate the shredder selection of these leaves in mixture treatments.

Fungal biomass accrual on leaves tends to increase the leaf N content, and the enzymatic maceration of leaves by the fungal community results in smaller and less refractory plant polymers, both processes making leaf resources more palatable to shredders [55,56]. Nevertheless, other studies show that a high fungal biomass does not necessarily imply a higher palatability of leaves, suggesting that shredder feeding depends on other characteristics, such as the leaf toughness, nutrient content, presence of mycotoxins and adaptation of shredders to those chemicals [43,57].

The similar consumption rates between A and B leaves did not translate to similar growth rates. The RGR and IGR were lower when only B leaf discs were offered. This result suggests that the consumption rate of B leaf discs was not sufficient to achieve similar growth. Consumers have two ways to compensate for the limitations of a poor resource quality, increasing consumption (feeding compensation [58]) or increasing assimilation rates, for example, by enhancing the retention time in their guts [59]. In general, the treatments with leaf mixtures also showed significantly lower growth rates regarding t1 with the exception of t5 for RGR. Our shredder mainly selected A leaf discs in mixture treatments, but the lower availability and/or the presence of less palatable leaf discs from stream B also limited its growth rate.

Food quality affects energy allocation (lipid storage). According to Flores et al. [58], the larvae fed poor-quality resources allocated a higher proportion of lipids to their body conditions than to growth. Larvae fed leaves of the poorest quality (from stream B) tended to allocate more lipids than larvae fed leaves of the richest quality (from stream A). Nevertheless, we did not find significant differences.

Finally, our results showed that the leaf quality affected the basal metabolism of the larvae. The basal metabolic rate determines the energetic cost of living, and after meeting the baseline energy requirements, shredders tend to allocate excess energy to other functions, such as growth and reproduction. The larvae fed leaf discs from stream B showed the highest oxygen consumption rate. This higher metabolism leads to lower energy being invested in growth, as shown in our results [59].

Consumers tend to maximize their feeding preferentially on food resources that are energetically most profitable [20]. They meet their elemental composition requirements to optimize their growth and reproduction, feeding preferentially on high-quality resources [60,61], and our findings are consistent with these statements. While the response of shredders has been strongly related to resource quality, what happens when high-quality resources are scarce remains in question. Other questions remaining include whether consumers actively search for high-quality resources even though they are the least abundant or whether they prefer to consume without selection and exert a more efficient assimilation to maintain homeostasis. Cruz-Rivera & Hay [62] suggested that resource selection seems to be related to the mobility of organisms. Our results suggest that when mobility is not a handicap, shredders seemed to actively select the food of better quality based on the quality properties despite its lower abundance, although this can limit their growth. We hope this finding stimulates future research to explore how mobility and resource availability interact in shredders.

In the context of increasing global water demand and aridification, flow intermittency will become more frequent, leading to drastic changes in food quality and quantity in rivers. Our findings demonstrate that such changes affect the fungal colonization of leaf litter, reducing several litter quality properties and ultimately affecting shredder consumption rates, growth and feeding selections. These responses could therefore potentially threaten the entire fluvial food web. These results provide a better understanding of the effects of changes in flow conditions on ecosystem functioning (leaf litter processing) in rivers and warn of the importance of guaranteeing the natural hydrological dynamics via a better management of water use.

Supporting information

S1 Table. Consumer consumption and growth data.
(PDF)

Acknowledgments

We gratefully acknowledge Julio C. López-Doval for his help in the lipid analyses, Veronica Granados for her help in the field, and especially Cayetano Gutierrez-Cánovas for his help with the statistical analyses in R.

We would like to thank two anonymous reviewers for constructive suggestions on earlier versions of the manuscript.

Author Contributions

Conceptualization: Rebeca Arias-Real, Margarita Menéndez, Isabel Muñoz.

Data curation: Rebeca Arias-Real, Margarita Menéndez, Meritxell Abril, Francesc Oliva, Isabel Muñoz.

Formal analysis: Rebeca Arias-Real, Margarita Menéndez, Francesc Oliva, Isabel Muñoz.

Investigation: Rebeca Arias-Real, Margarita Menéndez, Isabel Muñoz.

Methodology: Rebeca Arias-Real, Margarita Menéndez, Meritxell Abril, Isabel Muñoz.

Supervision: Margarita Menéndez, Meritxell Abril, Isabel Muñoz.

Validation: Rebeca Arias-Real, Margarita Menéndez, Isabel Muñoz.

Visualization: Rebeca Arias-Real, Margarita Menéndez, Isabel Muñoz.

Writing – original draft: Rebeca Arias-Real.

Writing – review & editing: Rebeca Arias-Real, Margarita Menéndez, Meritxell Abril, Francesc Oliva, Isabel Muñoz.

References

1. Datry T, Larned ST, Tockner K. Intermittent rivers: a challenge for freshwater ecology. Bioscience. 2014; 64: 229–235. https://doi.org/10.1093/biosci/bit027
2. Larned ST, Datry T, Arscott DB, Tockner K. Emerging concepts in temporary-river ecology. Freshw Biol. 2010; 55: 717–738. https://doi.org/10.1111/j.1365-2427.2009.02322.x
3. Martínez A, Pérez J, Molinero J, Sagarduy M, Pozo J. Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. Sci Total Environ. 2015; 503–504: 251–257. https://doi.org/10.1016/j.scitotenv.2014.06.018 PMID: 24962591
4. Leigh C, Boulton AJ, Courtwright JL, Fritz K, May CL, Walker RH, et al. Ecological research and management of intermittent rivers: an historical review and future directions. Freshw Biol. 2016; 61: 1181–1199. https://doi.org/10.1111/fwb.12646
5. Gonçalves AL, Chauvet E, Bärlocher F, Graça MAS, Canhoto C. Top-down and bottom-up control of litter decomposers in streams. Freshw Biol. 2014; 59: 2172–2182. https://doi.org/10.1111/fwb.12420
6. Abril M, Muñoz I, Menéndez M. Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. Sci Total Environ. 2016; 553: 330–339. https://doi.org/10.1016/j.scitotenv.2016.02.082 PMID: 26930306
7. Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, et al. Diversity meets decomposition. Trends Ecol Evol. 2010; 25: 372–380. https://doi.org/10.1016/j.tree.2010.01.010 PMID: 20189677
8. Kuehn KA. Lentic and lotic habitats as templets for fungal communities: Traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. Fungal Ecol. 2016; 19: 135–154. https://doi.org/10.1016/j.funeco.2015.09.009
9. Mas-Martí E, Romaní AM, Muñoz I. Consequences of warming and resource quality on the stoichiometry and nutrient cycling of a stream shredder. PLoS One. 2015; 10: 1–21. https://doi.org/10.1371/journal.pone.0118520 PMID: 25738818
10. Bärlocher F. Aquatic hyphomycetes in a changing environment. Fungal Ecol. 2016; 19: 14–27. https://doi.org/10.1016/j.funeco.2015.05.005
11. Grimmett IJ, Shipp KN, Macneil A, Bärlocher F. Does the growth rate hypothesis apply to aquatic hyphomycetes? Fungal Ecol. 2013; 6: 493–500. https://doi.org/10.1016/j.funeco.2013.08.002
12. Chung N, Suberkropp K. Effects of aquatic fungi on feeding preferences and bioenergetics of *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). Hydrobiologia. 2009; 630: 257–269. https://doi.org/10.1007/s10750-009-9820-y
13. Cargill AS, Cummins KW, Hanson BJ, Lowry RR. The role of lipids as feeding stimulants for shredding aquatic insects. Freshw Biol. 1985; https://doi.org/10.1111/j.1365-2427.1985.tb00215.x
14. Costantini ML, Rossi L. Species diversity and decomposition in laboratory aquatic systems: The role of species interactions. Freshw Biol. 2010; 55: 2281–2295. https://doi.org/10.1111/j.1365-2427.2010.02433.x
15. Gonçalves AL, Lírio A V., Graça MAS, Canhoto C. Fungal species diversity affects leaf decomposition after drought. Int Rev Hydrobiol. 2016; 101: 78–86. https://doi.org/10.1002/iroh.201501817
16. Mora-Gómez J, Elosegi A, Duarte S, Cássio F, Pascoal C, Romaní AM. Differences in the sensitivity of fungi and bacteria to season and invertebrates affect leaf litter decomposition in a Mediterranean stream. FEMS Microbiol Ecol. 2016; 92: 1–13. https://doi.org/10.1093/femsec/fiw121 PMID: 27288197
17. Covich A, Palmer M, Crowl T. The Role of Benthic Invertebrate Species in Freshwater Ecosystems—Zoobenthic Species Influence Energy Flows and Nutrient Cycling. Bioscience. 1999; 49: 119–127. https://doi.org/10.2307/1313537
18. Dieter D, Frindte K, Krüger A, Wurzbacher C. Preconditioning of leaves by solar radiation and anoxia affects microbial colonisation and rate of leaf mass loss in an intermittent stream. Freshw Biol. 2013; 58: 1918–1931. https://doi.org/10.1111/fwb.12180
19. Sanpera-Calbet I, Ylla I, Romaní AM, Sabater S, Muñoz I. Drought effects on resource quality in a Mediterranean stream: fatty acids and sterols as indicators. Limnetica. 2017; 36: 29–43. https://doi.org/10.23818/limn.36.03
20. Kaspari M, Donoso D, Lucas JA, Zumbusch T, Kay AD. Using nutritional ecology to predict community structure: a field test in Neotropical ants. Ecosphere. 2012; 3: 1–12. https://doi.org/10.1890/ES12-00136.1

21. Richardson JS, Zhang Y, Marczak LB. Resource subsidies across the land-freshwater interface and responses in recipient communities. River Res Appl. 2010; 26: 55–56. https://doi.org/10.1002/rra.1283
22. Marcarelli AM, Baxter CV, Mineau MM, Hall RO. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. Ecology. 2011; 92: 1215–1225. https://doi.org/10.1890/10-2240.1 PMID: 21797150
23. Frost PC, Benstead JP, Cross WF, Hillebrand H, Larson JH, Xenopoulos MA, et al. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecol Lett. 2006; 9: 774–779. https://doi.org/10.1111/j.1461-0248.2006.00919.x PMID: 16796566
24. Frost PC, Evans-White MA, Finkel Z V, Jensen TC, Matzek V. Are you what you eat? Physiological in an elementally world imbalanced stoichiometry. Oikos. 2005; 109: 18–28.
25. Menéndez M, Descals E, Riera T, Moya O. Effect of small reservoirs on leaf litter decomposition in Mediterranean headwater streams. Hydrobiologia. 2012; 691: 135–146. https://doi.org/10.1007/s10750-012-1064-6
26. Menéndez M, Descals E, Riera T, Moya O. Do non-native Platanus hybrida riparian plantations affect leaf litter decomposition in streams? Hydrobiologia. 2013; 716: 5–20. https://doi.org/10.1007/s10750-013-1539-0
27. Arsuffi TL, Suberkropp K. Leaf processing capabilities of aquatic hyphomycetes: interspecific differences and influence on shredder feeding preferences. Oikos. 1984; 42: 144–154. https://doi.org/10.2307/3544786
28. Suberkropp K, Arsuffi TL. Degradation, growth, and changes in palatability of leaves colonized by six aquatic hyphomycete species. Mycologia. 1984; 76: 398–407. https://doi.org/10.2307/3793320
29. Dang CK, Chauvet E, Gessner MO. Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. Ecol Lett. 2005; 8: 1129–1137. https://doi.org/10.1111/j.1461-0248.2005.00815.x PMID: 21352436
30. Gönczöl J, Révay À, Csontos P. Effect of sample size on the detection of species and conidial numbers of aquatic hyphomycetes collected by membrane filtration. Fundam Appl Limnol. 2001; 150: 677–691. https://doi.org/10.1127/archiv-hydrobiol/150/2001/677
31. Gessner MO. Ergosterol as a measure of fungal biomass. Methods to Study Litter Decomposition: A Practical Guide. 2005. pp. 231–236. https://doi.org/10.1007/1-4020-3466-0_25
32. Gessner MO, Chauvet E. Ergosterol-to-Biomass Conversion Factors for Aquatic Hyphomycetes. Appl Environ Microbiol. 1993; 59: 502–507. PMID: 16348874
33. Zöllner N, Kirsch K. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Z Gesamte Exp Med. 1962; 135: 545–561. https://doi.org/10.1007/BF02045455
34. Warkentin M, Freese HM, Karsten U, Schumann R. New and fast method to quantify respiration rates of bacterial and plankton communities in freshwater ecosystems by using optical oxygen sensor spots. Appl Environ Microbiol. 2007; 73: 6722–6729. https://doi.org/10.1128/AEM.00405-07 PMID: 17766446
35. Wang Y, Naumann U, Wright ST, Warton DI. Mvabund- an R package for model-based analysis of multivariate abundance data. Methods Ecol Evol. 2012; 3: 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x
36. Dufrene M, Legendre P. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr. 2011; 67: 345–366. https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2
37. Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol. 2010; 1: 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x
38. R Development Core Team R. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2011. https://doi.org/10.1007/978-3-540-74686-7
39. Suberkropp K, Arsuffi TL, Anderson JP. Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Appl Environ Microbiol. 1983; 46: 237–244. PMID: 16346343
40. Danger M, Chauvet E. Elemental composition and degree of homeostasis of fungi: Are aquatic hyphomycetes more like metazoans, bacteria or plants? Fungal Ecol. 2013; 6: 453–457. https://doi.org/10.1016/j.funeco.2013.05.007
41. Chamier A-C, Dixon PA. Pectinases in leaf degradation by aquatic hyphomycetes I: the field study. Oecologia. 1982; 52: 109–115. https://doi.org/10.1007/BF00349018 PMID: 28310115
42. Graça MAS, Maltby L, Calow P. Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* I: feeding strategies. Oecologia. 1993; 93: 139–144. https://doi.org/10.1007/BF00321203 PMID: 28313786

43. Graça MAS. The role of invertebrates on leaf litter decomposition in streams–a review. Int Rev Hydrobiol. 2001; 86: 383–393. https://doi.org/10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D

44. Pascoal C, Cássio F, Nikolcheva L, Bärlocher F. Realized fungal diversity increases functional stability of leaf litter decomposition under zinc stress. Microb Ecol. 2010; 59: 84–93. https://doi.org/10.1007/s00248-009-9567-z PMID: 19629577

45. Geraldes P, Pascoal C, Cássio F. Effects of increased temperature and aquatic fungal diversity on litter decomposition. Fungal Ecol. 2012; 5: 734–740. https://doi.org/10.1016/j.funeco.2012.05.007

46. Menéndez M, Descals E, Riera T, Moya O. Leaf litter breakdown in Mediterranean streams: effect of dissolved inorganic nutrients. Hydrobiologia. 2011; 669: 143–155. https://doi.org/10.1007/s10750-011-0657-9

47. Mas-Martí E, Sanpera-Calbet I, Muñoz I. Bottom-up effects of streambed drying on consumer performance through changes in resource quality. Aquat Sci. 2017; 79: 719–731. https://doi.org/10.1007/s00027-017-0531-6

48. Müller-Navarra DC, Brett MT, Liston AM, Goldman CR. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature. 2000; 403: 74–77. https://doi.org/10.1038/47469 PMID: 10638754

49. Torres-Ruíz M, Wehr JD, Perrone AA. Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. J North Am Benthol Soc. 2007; 26: 509–522. https://doi.org/10.1899/06-070.1

50. Torres-Ruíz M, Wehr JD. Changes in the nutritional quality of decaying leaf litter in a stream based on fatty acid content. Hydrobiologia. 2010; 651: 265–278. https://doi.org/10.1007/s10750-010-0305-9

51. Leberfinger K, Bohman I. Grass, mosses, algae, or leaves? Food preference among shredders from open-canopy streams. Aquat Ecol. 2010; 44: 195–203. https://doi.org/10.1007/s10452-009-9268-1

52. Arsuffi TL, Suberkropp K. Growth of two stream caddisflies (Trichoptera) on leaves colonized by different fungal species. J North Am Benthol Soc. 1986; 5: 297–305. https://doi.org/10.2307/1467482

53. Cargill AS, Cummins KW, Hanson BJ, Lowry RR. The role of lipids, fungi, and temperature in the nutrition of a shredder caddisfly, Clistoronia magnifica. Freshw Invertebr Biol. University of Chicago Press, Society for Freshwater Science; 1985; 4: 64–78. https://doi.org/10.2307/1467178

54. Bärlocher F, Kendrick B, Barlocher F. Assimilation efficiency of *Gammarus pseudolimnaeus* (Amphipoda) feeding on fungal mycelium or autumn-shed leaves. Oikos. 1975; 26: 55–59. https://doi.org/10.2307/3543277

55. Rong Q, Sridhar KR, Bärlocher F. Food selection in three leaf-shredding stream invertebrates. Hydrobiologia. 1995; 316: 173–181. https://doi.org/10.1007/BF00017435

56. Chauvet E, Cornut J, Sridhar KR, Selosse MA, Bärlocher F. Beyond the water column: Aquatic hyphomycetes outside their preferred habitat. Fungal Ecol. 2016; 19: 112–127. https://doi.org/10.1016/j.funeco.2015.05.014

57. Bärlocher F. The role of fungi in the nutrition of stream invertebrates. Bot J Linn Soc. 1985; 91: 83–94. https://doi.org/10.1111/j.1095-8339.1985.tb01137.x

58. Flores L, Larrañaga A, Elosegi A. Compensatory feeding of a stream detritivore alleviates the effects of poor food quality when enough food is supplied. Freshw Sci. 2014; 33: 134–141. https://doi.org/10.1086/674578

59. Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB. The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. Funct Ecol. 2015; 29: 479–486. https://doi.org/10.1111/1365-2435.12396

60. Sterner RW, Elser JJ. Ecological stoichiometry: the biology of elements from molecules to the biosphere. The New phytologist. 2002. https://doi.org/10.1111/j.1469-8137.2010.03214.x

61. Woodward G. Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. Freshw Biol. 2009; 54: 2171–2187. https://doi.org/10.1111/j.1365-2427.2008.02081.x

62. Cruz-Rivera AE, Hay ME, Ecology S. Can Quantity Replace Quality? Food Choice, Compensatory Feeding, and Fitness of Marine Mesograzers Published by: Ecological Society of America. Ecology. 2008; 81: 201–219.

Science of the Total Environment 703 (2020) 135485

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

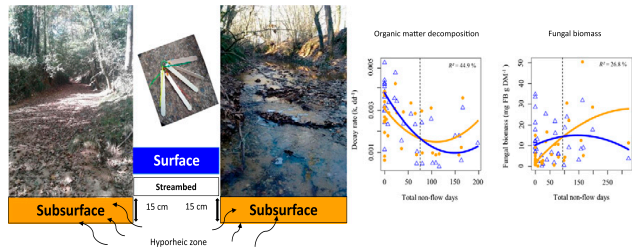
Rebeca Arias-Real [a,*], Isabel Muñoz [a], Cayetano Gutierrez-Cánovas [a,b,c], Verónica Granados [a], Pilar Lopez-Laseras [a], Margarita Menéndez [a]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, Universitat de Barcelona, Barcelona, Spain.
[b] Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.
[c] Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal.

HIGHLIGHTS

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

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 July 2019
Received in revised form 8 November 2019
Accepted 10 November 2019
Available online 18 November 2019

Editor: Sergi Sabater

Keywords:
Organic matter decomposition
Microhabitats
Flow intermittency
Fungal biomass
Hyporheic zone

ABSTRACT

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

* Corresponding author.
E-mail address: rebeca.arias.real@ub.edu (R. Arias-Real).

https://doi.org/10.1016/j.scitotenv.2019.135485
0048-9697/

1. Introduction

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

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

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

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

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

2. Methodology

2.1. Study area

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

2.2. Stream hydrology

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

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

2.3. Environmental factors

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

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

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

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

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

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

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

To determine the grain size distribution, fresh sediment samples (first aliquot) were first treated with H_2O_2 (10% volume) to remove organic matter and later disaggregated and dispersed ultrasonically with pyrophosphate. Fractions up to 2 mm were determined by sieving, while the determination of fractions below 2 mm was performed with a Beckman-Coulter LS230 laser. Then, the dry material (second aliquot) was ground using an agate mortar until it was completely homogenised, and we analysed the nitrogen (N) and carbon (C) concentrations using a Thermo Elemental Analyser 1108 (Thermo Scientific, Milan, Italy). We expressed the results in terms of C:N molar ratios.

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

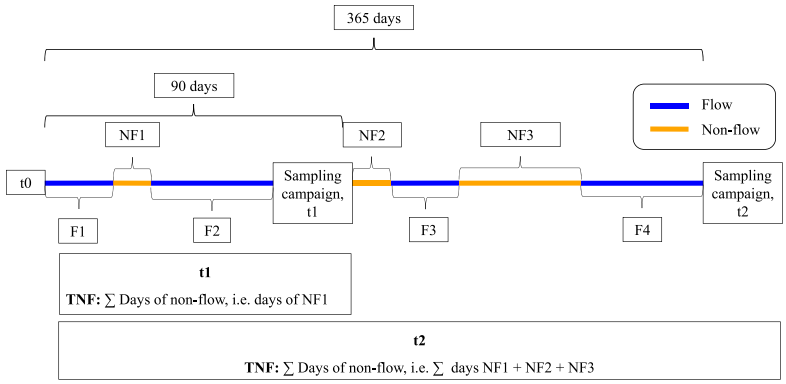


Fig. 1. Example of the calculation of total non-flow days (TNF) in this study. t0 is the time when the experiment started and the wood-sticks and loggers were placed on the streams, t1 is the time when we took the first wood-sticks and t2 is the time when we took the remaining wood-sticks and the experiment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

2.4. Organic matter experiment

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

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

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

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

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

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

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

2.5. Data analysis

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

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

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

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

To explore the relative importance of hydrology (TNF) and the best environmental predictors into the models, we performed variance

partitioning on LMM and LMs using the *variancePartition* R package (Hoffman and Schadt, 2016).

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

3. Results

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

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

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

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

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

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

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

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

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

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

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

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

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

4. Discussion

Overall, our findings confirm our hypothesis that subsurface processes had an important contribution to sustaining microbial decomposition during the non-flow periods of intermittent and ephemeral streams (IRES). We also showed that the magnitude of microbial decomposition and fungal biomass in the surface and subsurface zones depends on the local environmental factors of streams, such as SRP in the surface zone and sediment grain size, water content and sediment C:N ratio in the subsurface zone.

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

Previous research has shown that the duration of the non-flow period is a key factor in controlling microbial activity and OM decomposition (Bruder et al., 2011; Foulquier et al., 2015). However, thus far, most studies have focused on either the surface zone or the subsurface zone (Burrows et al., 2017; Corti and Drummond, 2011; Datry et al., 2018; Pinna and Basset, 2004), rather than simultaneously considering both

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

	Hydrological variables	SES	SE	*p*-Value	Explained variance	r^2_m	r^2_c
OM decay rate, k (dd^{-1})	Intercept	0.003	0.001	<0.001		44.9	73.6
	TNF	−0.001	0.000	**<0.001**	38.4		
	Zone	0.000	0.000	0.121	0.5		
	TNF x zone	−0.001	0.000	**0.002**	3.9		
	TNF²	−0.001	0.000	**0.032**	1.8		
Fungal Biomass (mg FB g DM^{-1})	Intercept	1.627	0.341	<0.001		26.8	65.1
	TNF	1.194	0.206	**<0.001**	2		
	Zone	0.697	0.228	**0.004**	4.2		
	TNF x zone	−1.125	0.229	**<0.001**	21.1		
	TNF²	0.883	0.233	**<0.001**	11.1		

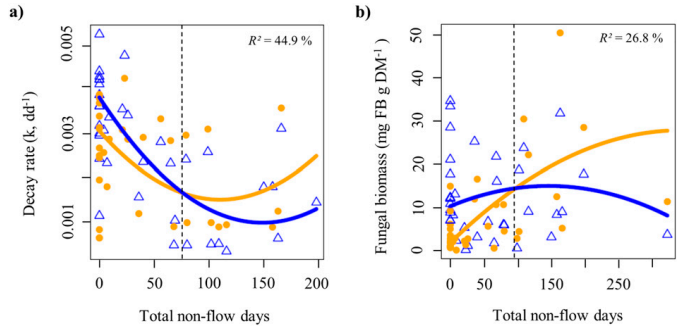


Fig. 2. Responses of decay rates (a) and fungal biomass (b) to TNF and their interaction with zone. Fitted lines are shown for the surface (blue) and subsurface (orange) zones in response to non-flow days. Vertical black bars show the temporal point where OM decomposition and fungal biomass in the subsurface zone become greater than the corresponding values at the surface.

zones. Nevertheless, our results demonstrate that simultaneously studying both zones is crucial to furthering our understanding of how microbial decomposition may respond to the expected increase in the duration and severity of flow intermittency as a result of climate change. In fact, although OM processing on both zones decreases with increased intermittency, the subsurface zone could maintain OM decomposition when the period without flow lengthens (Figs. 2a and 3a); for instance, streams with >75 days of non-flow could maintain approximately the same decomposition rates as the surface zones in streams with <75 days of non-flow.

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

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

Part of the explanation could be that the subsurface zone acts as a valuable refuge that maintains microbial activity, OM processing and nutrient cycling during non-flow periods (Marxen et al., 2010; Steward et al., 2012; Zoppini et al., 2014). The fact that the subsurface zone maintains an important number of active microbial organisms during the non-flow periods could translate into maintaining decomposition, as our results show that the subsurface zone had more constant or stable environmental conditions than the surface zone, during flow cessation. In addition, as this zone remains saturated with water for longer periods (Martínez et al., 2015), it provides a habitat for benthic organisms that move vertically into the subsurface zone, and it is the major compartment of OM storage (Boulton et al., 1899; Grimm and

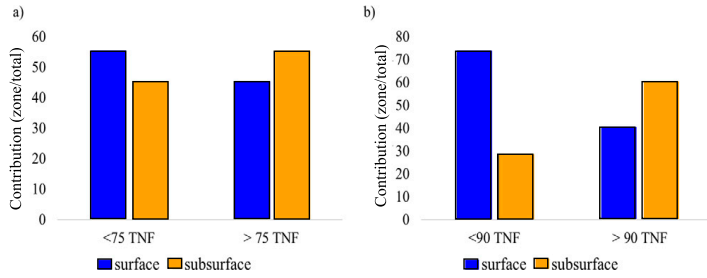


Fig. 3. Contribution of decay rates (a) and fungal biomass (b) relative to the total decay rates and fungal biomass, respectively (i.e., the sums of decay rates and fungal biomass in the surface and subsurface zones).

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

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

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

Fisher, 1984). In addition, groundwater inputs and bank inflows can help to keep the subsurface zone saturated for longer (Boulton et al., 1998; Burrows et al., 2017).

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

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

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

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

Fungal biomass is negatively linked to coarse sand content, which enables better hydraulic and vertical connectivity (Arce et al., 2019).

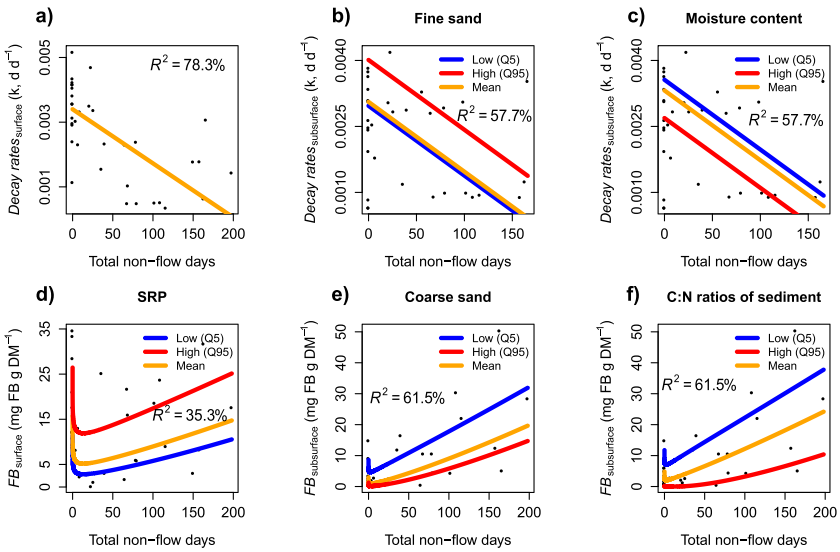


Fig. 4. Responses of OM decay rates (a, b, c) and fungal biomass (d, e, f) to hydrological and environmental predictors using LMM and LMs. Fitted lines are shown for OM decay rates and fungal biomass in response to total non-flow days. Different colours represent different levels (Q5, Q50, Q95) for the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

Nevertheless, when hydraulic connectivity disappears as flow ceases in the surface zone, water loss is faster with larger particle sizes, such as coarse sand, than with other sediments, such as fine sand (Mardhiah et al., 2014).

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

5. Conclusions

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

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

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

Declaration of competing interest

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

Acknowledgements

We gratefully acknowledge the field survey assistance of Giulia Gionchetta and Miriam Colls. We would like to thank Sergi Sabater and the three anonymous reviewers for constructive suggestions on earlier versions of the manuscript.

The Ministry of Economy, Industry and Competitiveness of Spain (MINECO) funded this work through projects: CGL2014-58760-C3-1-R (FUNSTREAM) and CGL2017-88640-C2-2-R (SPACESTREAM).

Rebeca Arias-Real held a predoctoral grant BES-2015-073961 (MINECO, FPI). CG-C was supported by a "Juan de la Cierva-Formación" research contract (MINECO, FJCI-2015-25785) and STREAMECO project (Biodiversity and ecosystem functioning under climate change: from the gene to the stream, POCI-01-0145-FEDER-029505), funded by Fundação para a Ciência e a Tecnologia (Portugal).

References

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.

Akaike, 1973. Information theory and an extension of themaximum likelihood principle. In: Petrov, B.N. (Ed.), Proceedings of the Second International Symposium on Information Theory. Akad. Kiado, Budapest, pp. 267–281.

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.

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 (12), e0, 1–17. https://doi.org/10.1371/journal.pone.0208272.

Arroita, M., Aristi, I., Flores, L., Larrañaga, A., Díez, J., Mora, J., Romaní, A.M., Elosegi, A., 2012. The use of wooden sticks to assess stream ecosystem functioning: comparison with leaf breakdown rates. Sci. Total Environ. 440, 115–122. https://doi.org/10.1016/j.scitotenv.2012.07.090.

Artigas, J., Romaní, A.M., Sabater, S., 2008. Effect of nutrients on the sporulation and diversity of aquatic hyphomycetes on submerged substrata in a Mediterranean stream. Aquat. Bot. 88, 32–38. https://doi.org/10.1016/j.aquabot.2007.08.005.

Barnard, R.L., Osborne, C.A., Firestone, M.K., 2015. 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.

Blazewicz, S.T.J.B., Chwartz, E.G.S., 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.

Boulton, A.A.J., Datry, T., Kasahara, T., Mutz, M., Jack, A., Boulton, A.J., Datry, T., Kasahara, T., Mutz, M., Stanford, J.A., 1899. Ecology and management of the hyporheic zone: stream – groundwater interactions of running waters and their floodplains. J. North Am. Benthol. Soc. 29, 26–40. https://doi.org/10.1899/08-017.1.

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.

Bruder, A., Chauvet, E., Gessner, M.O., 2011. Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency. Fuctional Ecol 25, 1269–1277. https://doi.org/10.1111/j.1365-2435.2011.01903.x.

Burrows, R.M., Rutlidge, H., Bond, N.R., Eberhard, S.M., Auhl, A., Andersen, M.S., Valdez, D.G., Kennard, M.J., 2017. High rates of organic carbon processing in the hyporheic zone of intermittent streams. Sci. Rep., 1–11 https://doi.org/10.1038/s41598-017-12957-5.

Constantz, J., Stonestrom, D., Stewart, A.E., Smith, T.R., 2001. Analysis of streambed temperatures in ephemeral channels to determine streamflow frequency and duration. Water Resour. Res. 37, 317–328. https://doi.org/10.1029/2000WR900271|.

Cornut, J., Arnaud, E., 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., 2541–2556 https://doi.org/10.1111/j.1365-2427.2010.02483.x.

Cornut, J., Elger, A., Greugny, A., Bonnet, M., Chauvet, E., 2012. Coarse particulate organic matter in the interstitial zone of three French headwater streams. Ann. Limnol. - Int. J. Limnol. 48, 303–313. https://doi.org/10.1051/limn/2012019.

Cornut, J., Chauvet, E., Mermillod-blondin, F., Assemat, F., Elger, A., 2014. Aquatic hyphomycete species are screened by the hyporheic zone of. 80, 1949–1960. https://doi.org/10.1128/AEM.03024-13.

Corti, R., Drummond, T.D.L., 2011. Natural variation in immersion and emersion affects breakdown and invertebrate1 colonization of leaf litter in a temporary river. Aquat. Ecol. 73, 537–550. https://doi.org/10.1007/s00027-011-0216-5.

Costantini, M.L., Rossi, L., 2010. Species diversity and decomposition in laboratory aquatic systems: the role of species interactions. Freshw. Biol. 55, 2281–2295. https://doi.org/10.1111/j.1365-2427.2010.02433.x.

Datry, T., Claret, R.C.C., Philippe, M., 2011. Flow Intermittence Controls Leaf Litter Breakdown in a French Temporary Alluvial River: The "'Drying Memory"'. , pp. 471–483. https://doi.org/10.1007/s00027-011-0193-8.

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.

Datry, T., Singer, G., Sauquet, E., Jorda-Capdevila, D., Schiller, D. Von, Magand, C., Pa, P., Miliša, M., Acuña, V., Alves, M.H., Augeard, B., Brunke, M., Cid, N., Csabai, Z., England, J., Froebrich, J., Koundouri, P., Lamouroux, N., Martí, E., Morais, M., Munné, A., Pesic, V., Previši, A., Reynaud, A., Robinson, C., Sadler, J., Skoulikidis, N., Terrier, B., Tockner, K., Vesely, D., Zoppini, A., 2017. Science and Management of Intermittent Rivers and Ephemeral Streams (SMIRES). Res. Ideas Outcomes 3, e21774. https://doi.org/10.3897/rio.3.e21774.

Datry, T., Foulquier, A., Corti, R., von Schiller, D., Tockner, K., Mendoza-Lera, C., Clément, J.C., Gessner, M.O., Moleón, M., Stubbington, R., Gücker, B., Albariño, R., Allen, 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.J., Boëchat, I.G., Boersma, K.S., Bogan, M.T., Bonada, N., Bond, N.R., Brintrup Barría, K.C., Bruder, A., Burrows, R.M., Cancellario, T., Canhoto, C., Carlson, S.M., Cauvy-Fraunié, S., Cid, N., Danger, M., de Freitas Terra, B., De Girolamo, A.M., de La Barra, E., del Campo, R., Diaz-Villanueva, V.D., Dyer, F., Elosegi, A., Faye, E., Febria, C., Four, B., Gafny, S., Ghate, S.D., Gómez, R., Gómez-Gener, L., Graça, M.A.S., Guareschi, S., Hoppeler, F., Hwan, J.L., Jones, J.I., Kubheka, S., Laini, A., Langhans, S.D., Leigh, C., Little, C.J., Lorenz, S., Marshall, J.C., Martín, E., McIntosh, A.R., Meyer, E.I., Miliša, M., Mlambo, M.C., Morais, M., Moya, N., Negus, P.M., Niyogi, D.K., Papatheodoulou, A., Pardo, I., Pařil, P., Pauls, S.U., Pešić, V., Polášek, M., Robinson, C.T., Rodríguez-Lozano, P., Rolls, R.J., Sánchez-Montoya, M.M., Savić, A., Shumilova, O., Sridhar, K.R., Steward, A.L., Storey, R., Taleb, A., Uzan, A., Vander Vorste, R., Waltham, N.J., Woelfle-Erskine, C., Zak, D., Zarfl, C., Zoppini, A., 2018. A global analysis of terrestrial plant litter dynamics in non-perennial waterways. Nat. Geosci. 11, 497–503. https://doi.org/10.1038/s41561-018-0134-4.

Duarte, S., Mora-Gómez, J., Romaní, A.M., 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.

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.

Follstad Shah, J.J., Kominoski, J.S., Ardón, M., Dodds, W.K., Gessner, M.O., Griffiths, N.A., Hawkins, C.P., Johnson, S.L., Lecerf, A., LeRoy, C.J., Manning, D.W.P., Rosemond, A.D., Sinsabaugh, R.L., Swan, C.M., Webster, J.R., Zeglin, L.H., 2017. Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. Glob. Chang. Biol. 23, 3064–3075. https://doi.org/10.1111/gcb.13609.

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.

Gessner, M.O., 2005. Ergosterol as a measure of fungal biomass. Methods to Study Litter Decomposition: A Practical Guide, pp. 231–236. https://doi.org/10.1007/1-4020-3466-0_25.

Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. 59, 502–507.

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., Chauvet, E., Dobson, M., 1999. A perspective on leaf litter breakdown in streams. Oikos 85, 377–384. https://doi.org/10.2307/3546505.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends Ecol. Evol. 25, 372–380. https://doi.org/10.1016/j.tree.2010.01.010.

Ghate, S.D., Sridhar, K.R., 2015. Diversity of aquatic hyphomycetes in streambed sediments of temporary streamlets of Southwest India. Fungal Ecol. 14, 53–61. https://doi.org/10.1016/j.funeco.2014.11.005.

Gionchetta, G., Oliva, F., Menéndez, M., Lopez, P., Anna, L., 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.

Gonçalves, A.L., Chauvet, E., Bärlocher, F., Graça, M.A.S., Canhoto, C., 2014. Top-down and bottom-up control of litter decomposers in streams. Freshw. Biol. 59, 2172–2182. https://doi.org/10.1111/fwb.12420.

Gonçalves, A.L., Lírio, A.V., Graça, M.A.S., Canhoto, C., 2016. Fungal species diversity affects leaf decomposition after drought. Int. Rev. Hydrobiol. 101, 78–86. https://doi.org/10.1002/iroh.201501817.

Graça, M.A.S., Cressa, C., Gessner, M.O., Feio, M.J., Callies, K.A., Barrios, C., 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Freshw. Biol. 46, 947–957. https://doi.org/10.1046/j.1365-2427.2001.00729.x.

Grimm, N.B., Fisher, S., 1984. Exchange between interstitial and surface water: implications for stream metabolism and nutrient cycling. Hydrobiologia 111, 219. https://doi.org/10.1007/BF00007202.

Krauss, Gudrun, Schlosser, D., Wesenberg, D., Gerd-joachim, Krauss, Sol, M., Bärlocher, F., 2011. Fungi in freshwaters: ecology, physiology and biochemical potential. FEMS Microbiol. Rev. 35, 620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x.

Harjung, A., Perujo, N., Butturini, A., Romaní, A.M., Sabater, F., 2019. Responses of microbial activity in hyporheic pore water to biogeochemical changes in a drying headwater stream. Freshw. Biol. https://doi.org/10.1111/fwb.13258 fwb.13258.

Hoffman, G.E., Schadt, E.E., 2016. variancePartition: interpreting drivers of variation in complex gene expression studies. BMC Bioinformatics 17, 483. https://doi.org/10.1186/s12859-016-1323-z.

Kuehn, K.A., 2016. Lentic and lotic habitats as templets for fungal communities: traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. Fungal Ecol. 19, 135–154. https://doi.org/10.1016/j.funeco.2015.09.009.

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|.

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.

Mardhiah, U., Caruso, T., Gurnell, A., Rillig, M.C., 2014. Soil Biology & Biochemistry Just a matter of time : fungi and roots significantly and rapidly aggregate soil over four decades along the Tagliamento River , NE Italy. Soil Biol. Biochem. 75, 133–142. https://doi.org/10.1016/j.soilbio.2014.04.012.

Martínez, A., Pérez, J., Molinero, J., Sagarduy, M., Pozo, J., 2015. Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. Sci. Total Environ. 503–504, 251–257. https://doi.org/10.1016/j.scitotenv.2014.06.018.

Marxen, 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.

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.

Menéndez, M., Descals, E., Riera, T., Moya, O., 2011. Leaf litter breakdown in Mediterranean streams: effect of dissolved inorganic nutrients. Hydrobiologia 669, 143–155. https://doi.org/10.1007/s10750-011-0657-9.

Mora-gómez, J., 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.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Mustonen, K.R., Mykrä, H., Louhi, P., Markkola, A., Tolkkinen, M., Huusko, A., Alioravainen, N., Lehtinen, S., Muotka, T., 2016. Sediments and flow have mainly independent effects on multitrophic stream communities and ecosystem functions. Ecol. Appl. 26, 2116–2129. https://doi.org/10.1890/15-1841.1/suppinfo.

Petersen, R., Cummins, K., 1974. Leaf processing in a woodland stream. Freshw. Biol. 4, 343–368. https://doi.org/10.1111/j.1365-2427.1974.tb00103.x.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2017. Nlme: Linear and Nonlinear Mixed Effects Models_. R Packag. Version 3.1–131 2017.

Pinna, M., Basset, A., 2004. Summer drought disturbance on plant detritus decomposition processes in three River Tirso (Sardinia, Italy) sub-basins. Hydrobiologia 522, 311–319. https://doi.org/10.1023/B:HYDR.0000029984.93471.b5.

Pohlon, E., Fandino, A.O., Marxen, J., 2013. 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.

R Development Core Team, R, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing https://doi.org/10.1007/978-3-540-74686-7.

Reardon, J., Company, C.M., Valley, S., 1969. United States Patent Office 3–6.

Sanpera-Calbet, I., Acuña, V., Butturini, A., Marcé, R., Muñoz, I., 2016. El Niño southern oscillation and seasonal drought drive riparian input dynamics in a Mediterranean stream. Limnol. Oceanogr. 61, 214–226. https://doi.org/10.1002/lno.10211.

Scott, S., Zhang, Y., 2012. Contrasting effects of sand burial and exposure on invertebrate colonization of leaves. Am. Midl. Nat. 167, 68–78. https://doi.org/10.1674/0003-0031-167.1.68.

Solagaistua, L., Arroita, M., Aristi, I., Larrañaga, A., Elosegi, A., 2016. Changes in discharge affect more surface than subsurface breakdown of organic matter in a mountain stream. Mar. Freshw. Res. 67, 1826–1834.

Sridhar, K.R., Duarte, S., Cássio, F., Pascoal, C., 2009. The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. Int. Rev. Hydrobiol. (4), 399–409 https://doi.org/10.1002/iroh.200811154.

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.

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|.

Stout, R.J., 1989. Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach. Can. J. Fish. Aquat. Sci. 46, 1097–1106. https://doi.org/10.1139/f89-142.

Stubbington, R., 2012. The hyporheic zone as an invertebrate refuge: a review of variability in space , time , taxa and behaviour. Mar. Freshw. Res. 63, 293–311. https://doi.org/10.1071/MF11196.

Suberkropp, K., Arsuffi, T.L., Anderson, J.P., 1983. Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Appl. Environ. Microbiol. 46, 237–244.

Suberkropp, K., Gulis, V., Rosemond, A.D., Benstead, J.P., 2010. Ecosystem and physiological scales of microbial responses to nutrients in a detritus-based stream : results of a 5-year continuous enrichment. Limnol. Oceanogr. 55, 149–160.

Zoppini, A., Ademollo, N., Amal, 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., Leno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer https://doi.org/10.1007/978-0-387-87458-6.

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. 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.