Stream Biofilm Responses to Flow Intermittency: From Cells to Ecosystems

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Temporary streams are characterized by the alternation of dry and wet hydrological phases, creating both a harsh environment for the biota as well as a high diversity of opportunities for adaptation. These systems are mainly microbial-based during several of these hydrological phases, and those growing on all solid substrata (biofilms) accordingly change their physical structure and community composition. Biofilms experience large decreases in cell densities and biomass, both of bacteria and algae, during dryness. Algal and bacterial communities show remarkable decreases in their diversity, at least locally (at the habitat scale). Biofilms also respond with significant physiological plasticity to each of the hydrological changes. The decreasing humidity of the substrata through the drying process, and the changing quantity and quality of organic matter and nutrients available in the stream during that process, causes unequal responses on the biofilm bacteria and algae. Biofilm algae are affected faster than bacteria by the hydric stress, and as a result the ecosystem respiration resists longer than gross primary production to the increasing duration of flow intermittency. This response implies enhancing ecosystem heterotrophy, a pattern that can be exacerbated in temporary streams suffering of longer dry periods under global change.

Keywords: biofilm, dry-rewetting cycle, temporary, intermittency, bacteria, algae, Mediterranean
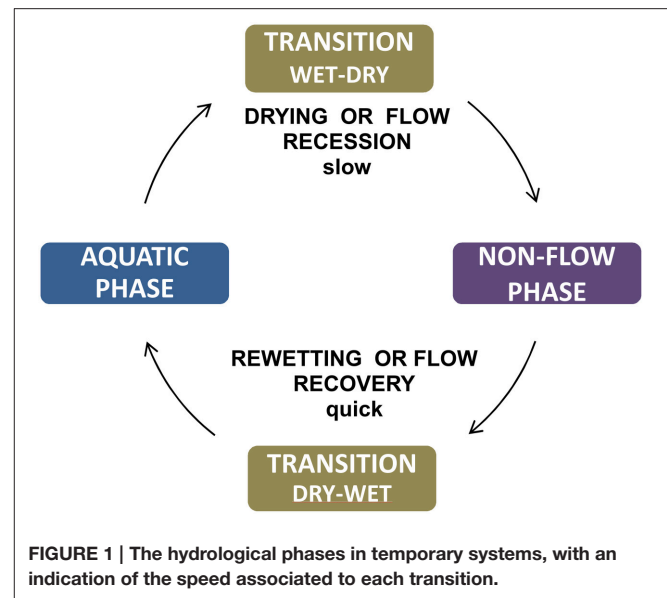
THE RELEVANCE OF TEMPORARY STREAMS IN THE WORLD

Flow intermittency is part of the natural hydrology for streams and rivers, especially in arid and semi-arid landscapes. Temporary streams, or those that cease to flow at some points in space and time along their course, are a large fraction of many river networks, basically in tributaries of small order, but also in segments in the middle and lower parts of river networks. The number of temporary streams has been probably underestimated (Acuña et al., 2014), as it appears from the application of novel on-site sensors, advances in remote sensing, and new modeling approaches. Flow intermittency has been estimated to account for 69% of first-order streams below 60°, and up to 34% of larger order rivers (Raymond et al., 2013). Flow intermittency produces periodic or event-related loss of hydrologic connectivity between stream compartments, with consequences for all the processes ongoing in a waterway. Thus, flow intermittency triggers a chain of cascading effects eventually affecting water chemistry and biogeochemistry, as well as biodiversity, and ultimately community structure and ecosystem functioning.

The direct implications of climate change and anomalous climatic events on flow regime are behind of the increasing temporality of many streams and rivers. Complex planetary scale processes, as well as local processes, may justify these situations. Anomalies such as the El Niño

episodes are associated with intense droughts in large areas of the Planet. The driest period in history in large rivers such as the Magdalena and Cauca (Colombia) has been recently reported because of the El Niño influence. The Mediterranean basin is one of the most prominent "hot spots" for potential changes (Giorgi and Lionello, 2008). Most models agree that climate change will largely affect the area, and that this will be expressed by more intense summer droughts, higher temperatures, and more frequent episodes of intense rainfall. Similar changes will probably influence most Mediterranean-type regions worldwide (Bonada and Resh, 2013). In all of them, an obvious consequence of these changes is the enhancement of water flow intermittency. It is also well-known that some rivers are shifting from permanent to temporary because of unsustainable water extraction (Barceló and Sabater, 2010; Larned et al., 2010). Such a mixture of natural climate irregular patterns and human-driven components of global change may be particularly relevant in systems where the seasonal and interannual variability is high (as in Mediterranean systems; Gasith and Resh, 1999). The resulting altered hydrology of these systems will produce longer episodes of drying that will challenge the current status of their biological communities and of their ecosystem functioning.

The wax and wane of water makes the temporary streams rather unique. The two processes, drying and rewetting, altogether create a high diversity of situations in a rather short time (**Figure 1**). When the discharge decreases, the residence time of surface water increases, thereby leading to an average "ageing" of water (Vörösmarty and Sahagian, 2000). Decreased water flow may therefore lead to the transformation of the habitat character of rivers in the direction from lotic (moving waters) to lentic (standing waters), a process that can be termed as lentification (Sabater, 2008), usually associated with higher water temperatures, greater evaporative losses, and relevant changes in the biogeochemical processes as well as in the biological community inhabiting the river. This drying may continue for longer or shorter periods of time until there is complete flow interruption, when the water vanishes and there is complete streambed desiccation.

The drying operates in both longitudinal and vertical dimensions. Under continued basal flow decrease, flow interruption proceeds steadily, first by a depletion of superficial waters more or less accelerated by evaporation and infiltration, causing shallow surface habitats such as riffles to disappear, and the creation of a series of fragmented pools. In this case, named as the superficial dryness, pools may persist and remain together with a low water flow in the hyporheos (Lake, 2003). During a subsurface dryness even the hyporheic zone can dry out (Boulton, 2003), affecting the potential refuge for many organisms. And in an even more advanced step, or deep dry phase, the phreatic layer can descend causing the complete hydrological isolation of the river compartments and the early leaf abscission of riparian woody plants (Sabater et al., 2001). The velocity and intensity of the dryness process may depend on both natural and non-natural processes, and the outcome may therefore be very different. In case this extended decrease of the water table remains for long time, the riparian vegetation

FIGURE 1 | The hydrological phases in temporary systems, with an indication of the speed associated to each transition.

may be impaired and even disappears. In this case, effects may even extend to the soil moisture retention. This shorter or longer drying process is interrupted by a rewetting process with the flow return (**Figure 1**), usually faster than the drying episode and associated to very intense recovery processes of biogeochemical and biological processes (Romani and Sabater, 1997). Before the subsuperficial drought comes to an end, humidity can persist longer in the lower hyporheic layers, creating strong redox gradients between the superficial and subsuperficial layers.

The drying of sediments results in an increased sediment affinity for phosphorus and reduced availability to biota, enhanced nitrate content of the sediment (result of net nitrogen mineralization and net nitrification), and the inhibition of denitrification (Gómez et al., 2012). A further expression of the high mineralization activity is that dry stream beds may become net exporters of CO_2 as occurs in dry soils do (von Schiller et al., 2014). These processes revert on the return of flow to the stream. Aerobic penetration increases and anaerobic zones are then aerobic, and re-wetted sediments liberate phosphorus and nitrogen as a consequence of dying-induced microbial cell lysis (Baldwin and Mitchell, 2000). The organic matter accumulated in the streambed during the dry period, that has been the object of slow mineralization, is suddenly mobilized by the circulating waters. The water return to a previously dry stream bed is a "hot biogeochemical moment" (McClain et al., 2003), when biogeochemical reactions restart or accelerate after long quiescent periods, and both respiration and primary productivity are enhanced instream. These biogeochemical phases offer a variety of ecological niches for fast-growing, well adapted organisms, especially to those that may be favored by repeated drying-wetting cycles.
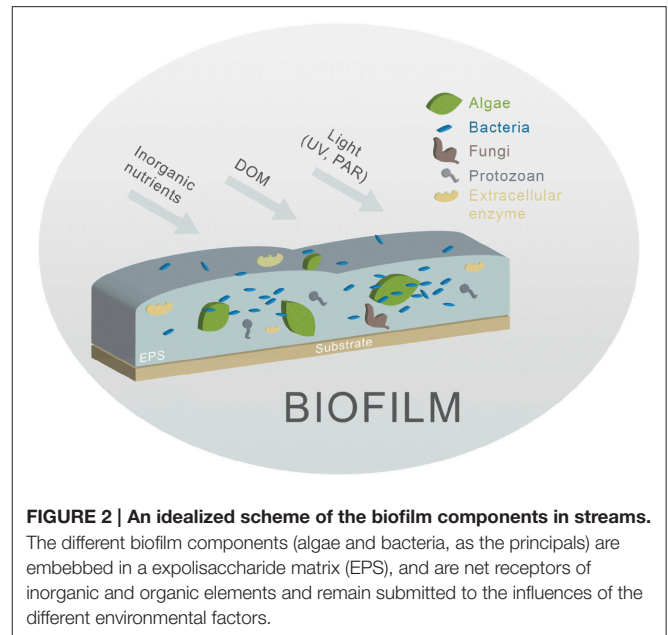
BIOFILMS IN TEMPORARY STREAMS

The general understanding of the response of biological communities to flow interruption is that they are formed by

subsets of communities inhabiting the permanent reaches, and that they usually contain a community of ubiquitous species with high dispersal capacity (Arscott et al., 2010; Datry et al., 2011). Investigations of biofilms in intermittent systems agree with this overall perspective, and have shown that bacterial communities are subsets of the community that inhabits the stream under usual water flow conditions (Zeglin et al., 2011; Timoner et al., 2014a). But beyond this general agreement, the ability of biofilms to resist desiccation, as well as their opportunism in colonizing the substrata after flow return, become very important features in temporary systems.

Biofilms are one of the main biological interphases in river ecosystems elsewhere, and probably the most important in intermittent rivers, where the importance of the water column is reduced during extended periods of the hydrological cycle. Biofilms have to be understood as biological consortia of autotrophs and heterotrophs, coexisting in a matrix of hydrated extracellular polymeric substances (EPS). These two main biological components are respectively mainly algae and cyanobacteria on one side, and bacteria and fungi on the other (**Figure 2**). Micro- and meiofauna also inhabit the biofilm, predating on the organisms and organic particles and contributing to its evolution and dispersal (Majdi et al., 2012). Biofilms therefore form a highly active biological consortium, ready to use organic and inorganic materials from the water phase, and also ready to use light or chemical energy sources. The EPS immobilize the cells and keep them in close proximity allowing for intense interactions including cell-cell communication and the formation of synergistic consortia (Flemming and Wingender, 2010). The EPS is able to retain extracellular enzymes and therefore allows the utilization of materials from the environment and the transformation of these materials into dissolved nutrients for the use by algae and bacteria. At the same time, the EPS contributes to protect the cells from desiccation as well from other hazards (e.g., biocides, UV radiation, etc.) from the outer world. On the other hand, the packing and the EPS protection layer limits the diffusion of gases and nutrients, especially for the cells far from the biofilm surface, and this limits their survival and creates strong gradients within the biofilm. Both the biofilm physical structure, and the plasticity of the organisms that live within it, ensure and support their survival in harsh environments or under changing environmental conditions.

The key for adaptation for microbial organisms may come from the ability to persist in (or colonize to) any of the potential refuges in the stream, and require their ability to recover and spread after flow returns. There are different potential refuges for biofilms in a drying stream. Perennial pools, leaf litter packs, or even subsuperficial sediments, may temporarily offer wet environments, therefore acting as refuges for microbes. These might suffice while humidity remains, but the prevalence of microbes depends then on the severity of the drought: no refuges remain in systems where desiccation becomes very long and harsh. The alternatives therefore are entering dormancy until the conditions become again favorable, or being able to colonize the particular habitat from the outside.

FIGURE 2 | An idealized scheme of the biofilm components in streams. The different biofilm components (algae and bacteria, as the principals) are embebbed in a expolisaccharide matrix (EPS), and are net receptors of inorganic and organic elements and remain submitted to the influences of the different environmental factors.

During the stream contraction process, pools are formed in the deeper parts of the stream, where lotic communities are gradually replaced by lentic others and even semiaquatic or terrestrially-derived biota (Stanley et al., 1997; Steward et al., 2012; Timoner et al., 2014a). In these pools, attached communities are progressively replaced by free-living (tycho-planktonic; Fazi et al., 2013) communities, and finally by subaerial communities when the drying is complete. And since the drying is not uniform, patches may occur along the stream, with lotic, lentic and terrestrial compartments, therefore providing a β-diversity that is higher than expected (Tornés and Sabater, 2010; Datry et al., 2014). Pools tend to disappear in the later phases of the stream desiccation, and therefore the dominant substrata have to be seen as the main source of refuge, and not so much the pools for the biofilms. As an example, dry biofilm on cobbles may confer protection to the cells that remain beneath (**Figures 3A,B**), resembling the biological soil crusts in arid regions (Belnap and Lange, 2003). Further, there is a difference between the organisms deriving from remnant pools and those deriving from the main substrata (Robson et al., 2008). Algae and bacteria in the biofilms may shift in their community composition in the same manner as other biological groups during the drying phase.

Adapting to these shrinking conditions is done at the expense of decreasing the local (alpha) diversity. The microbial assemblages in intermittent streams are formed communities of low diversity. This was defined by Tornés and Ruhí (2013) in an analysis of the diatom communities in different sets of permanent and intermittent streams. They showed that intermittent sites were the most species-poor, and with a lower proportion of specialist taxa than in permanent streams. This apparent poverty in species may be related to the few pioneer species that are able to colonize empty spaces once they are rewetted, and that are ubiquitous and generalist organisms able to colonize
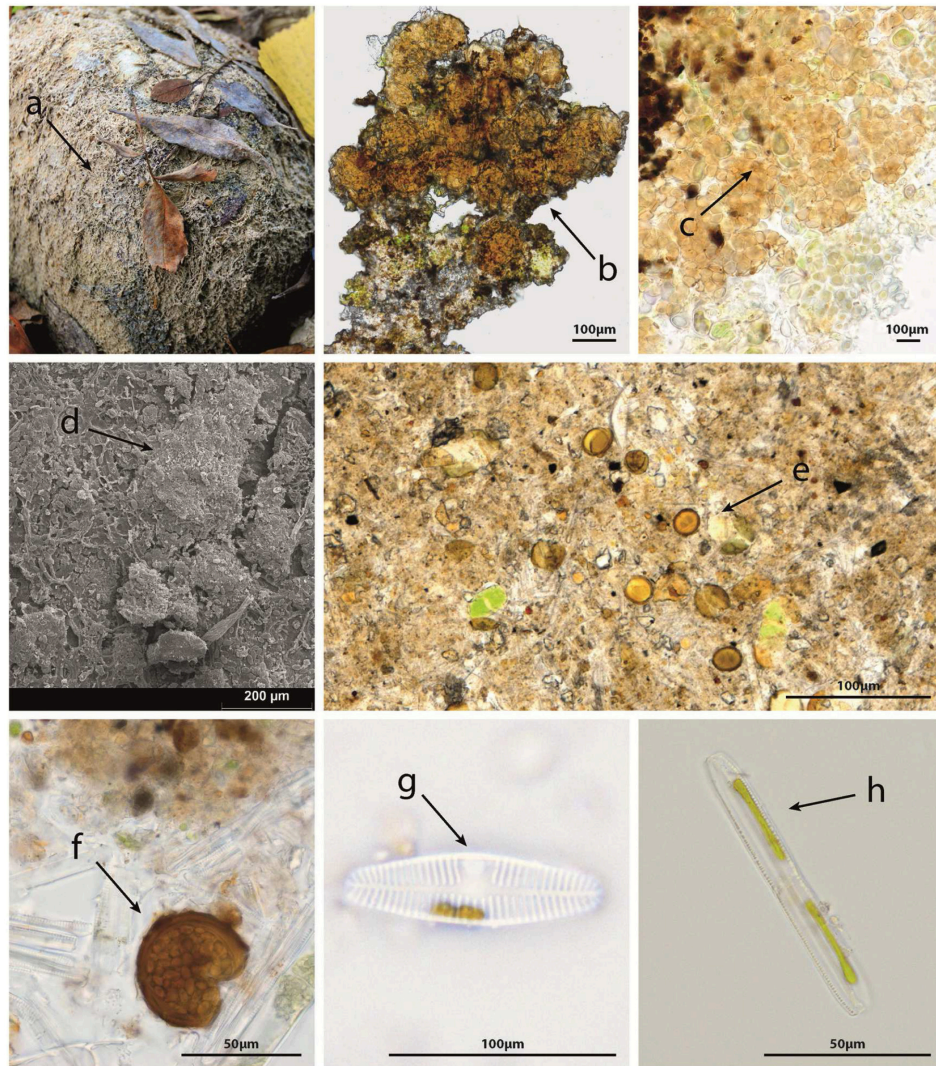
FIGURE 3 | Cell resistance structures in biofilms during the non-flow phase. Structures observed in the epilithon. **(A)** Bleached dry biofilm, **(B)** general view of the crust formation, **(C)** enlarged membrane of encrusting algae and change in color, **(D)** SEM image of the crust; **(E)** spores; **(F)** a resistant spore of *Oedogonium* sp. Adaptations in the epipsammon and hyporheos: **(G,H)** chloroplast reduction in diatom cells.

the changing environment. In the case of diatoms, particularly the genera *Achnanthes, Fragilaria,* and *Nitzschia* may attach to empty substrata in a few days (Sabater and Romani, 1996). Some members of family *Sphingomonadaceae* have also been reported to pioneer biofilm formation (Besemer et al., 2009). Pohlon et al. (2013) observed that a pioneer bacterial community dominated by members of the Cytophaga–Flavobacterium group settled onto the substratum within 12 h, and progressively incorporated Gammaproteobacteria and Betaproteobacteria. A decrease in bacterial diversity during non-flow may affect up to half of the Operational Taxonomic Units (OTUs) present (Timoner et al., 2014a), and affiliated taxa related to *Firmicutes, Actinobacteria,* and *Alphaproteobacteria* incorporated on the bacterial communities.

STRUCTURAL CHANGES AND ADAPTATIONS OF BIOFILMS TO DRY AND WET PHASES

Cell densities and biomass of bacteria and algae in the biofilm become highly affected during intermittency, though their patterns are different. Timoner et al. (2012) showed that drying in a Mediterranean stream caused a major decrease in autotrophic biomass, while the heterotrophic biomass was less affected. Algal biomass decreased up to 80% with streambed desiccation, but bacterial cell density decreased only by 20%. They suggested that the lower resistance of autotrophs than heterotrophs can be attributed to the physical conditions in the different stream compartments. Sand sediments preserve humid

conditions enough for the survival of bacteria (Mckew et al., 2010), but not so much for the algae that are light-limited there. The absence of water directly affects the performance of algal photosynthesis; however, some bacterial groups have the ability to remain active under low humidity conditions. The recovery of biomass may also follow different patterns for the different substrata; the epilithic biofilm can be the least resistant because it is the most exposed, but also the quicker to recover after flow return both in terms of autotrophs and heterotrophs (Timoner et al., 2012).

The velocity and intensity of drying determines the structural responses in the biofilms. Drying is a disturbance that causes a transition toward a new steady state in the biofilm structure (Barthès et al., 2015). Excessively rapid, or unanticipated, drying prevents the formation of resistance structures (Stanley et al., 2004), and random processes may be then more important for the organisms survival. In systems suffering such a unexpected drying, recovery will be mainly based on the recolonization from other areas of the stream where water still persists. On the contrary, progressive drying favors algal or bacterial species able to form resistance structures. Most stream biofilms show such structures in rivers traditionally affected by intermittency, indicating an unequivocal strategy toward dormancy on the dry beds until water resumes, which quickly re-activates microorganisms. The algae able to withstand desiccation show different strategies including the production of akinetes, cists, or protected eggs (**Figure 3**), favoring prevailing growth-forms, physiological responses, or even genetic adaptations. Mucilage-forming species may act as protectors of the living cells. This occurs in the diatom genera *Cymbella* and *Gomphonema*, which form stalks or tubes where the cells host. Diatoms are also capable of migrating from superficial to deeper sediments, as another survival strategy (Mckew et al., 2010). However, diatoms do not resist complete drying, and other algal groups showing cells with thick walls, such as Rhodophyta and Phaeophyta, are better able to resist the desiccation. This is the case of the red alga *Hildenbrandia rivularis*, which is able to survive long periods completely dry and immediately recover its photosynthetic activity after flow returns. *Hildenbrandia rivularis* is an encrusting species, able to attach extremely tight to the substrata, usually under low light irradiance and associated low growth rates. Encrusting forms are not unique to intermittent systems, but are a very effective persistence strategy. The green alga *Gongrosira* is another encrusting species even able to corrode the substrata onto which it establishes, able to persist after very long desiccation though at the cost of losing a high number of viable cells (Ledger et al., 2008). Many cyanobacteria are also extremely quick to react to humidity or water return, especially those having sheaths that quickly rehydrate. In calcareous streams, these may form stromatolitic-like forms that are very porous (Sabater, 2000) and highly persistent under complete dryness. Since one of the main concerns for the algae living in these environments is the genetic drift, some have developed polyploidy, with several packages of chromosomes (Nichols, 1980). This is the strategy of filamentous green algae such as *Oedogonium*, *Zygnema*, or *Spirogyra*, that are also able to form resistant sexual eggs, or zygospores, which are dormant

until favorable conditions occur (Stanley et al., 2004). Some of these filamentous species are also adapted to drift, and remain unattached, therefore able to move following the shrinking flow in the stream down to pools, where they can survive and recolonize the system afterwards.

Desiccation severely affects bacterial communities both in terms of abundance and diversity. During periods of low- to no-surface water, communities with a higher number of OTUs shift to communities with lower richness (e.g., with fewer OTUs; Amalfitano et al., 2008; Timoner et al., 2014a). Timoner et al. (2014a) found that Firmicutes, a bacterial phylum that encompasses many endospore-forming genera, temporarily replaced Cyanobacteria in epilithic biofilms during the non-flow period in a temporary Mediterranean stream. Additionally, many members of the Firmicutes possess multiplicity of rRNA operons in their genomes, an advantageous trait under conditions requiring rapid responses to resources availability (Klappenbach et al., 2001). A similar behavior was found for members of the Actinobacteria (Timoner et al., 2014a). Both Firmicutes and Actinobacteria have a gram-positive cell wall type that might favor resistance to desiccation in temporary streams. When a stream dries up, the hydrological fragmentation and the occurrence of pools favor the gradual reduction of bacterial alpha-diversity, as well as a change in the community composition. This was observed by Fazi et al. (2013) in an intermittent Mediterranean stream, where the bacterioplankton community composition (mostly Betaproteobacteria and Actinobacteria) in isolated pools changed according to redox shifts and quality of the DOM in the pools. Amalfitano et al. (2008) related the changes in community composition and their associated use of organic carbon to the sediment water content. They showed that only 14% of the initial living bacterial biomass persisted, but that was sufficient to reactivate the aquatic microbial food web after the arrival of new water.

Surface drying is followed by the hyporheic drying, though at a lower velocity, related to the grain size of the sediment and its hydraulic conductivity. This has implications for the resistance of the autotrophs, that in spite of the limiting light conditions in the hyporheos, can maintain some of their chlorophyll-a if hosted there during non-flow (Timoner et al., 2012). Since the velocity at which the sediment dries out is difficult to predict, irregular patches are produced according to the prevailing water flow paths or resistance to dry out. Febria et al. (2012) showed that surficial microbial communities could remain disconnected from those in the hyporheic, and the drying out of surface water affected interstitial pore water communities to a greater extent than those in the hyporheic sediment. The former showed a transient community, while those inhabiting the hyporheic sediment showed a more permanent composition. Even in these circumstances, bacteria may travel through the pore water and the hyporheic sediments (Febria et al., 2012), showing that these sediments may act as a type of refuge from desiccation for both bacteria and algae.

The bacterial community residing on cobbles may suffer the most abrupt changes of all other compartments, because they are the most exposed to desiccation, extreme air temperatures,

and major colonization by terrestrial microbes during the non-flow phase. The non-flow phase not only may reduce the richness and diversity of bacterial communities, but also may provide new habitat opportunities for certain bacterial taxa. Recently, Ruiz-González et al. (2015) showed that the highest contribution of bacteria from terrestrial sources was observed in smaller streams, as a consequence of the physical proximity, and decreased with the increasing size of the stream. It is therefore not surprising that temporary streams, that become fully terrestrial during a certain period of their hydrological cycle, may show such an influence. It is also not surprising that these effects may be particularly obvious in the epilithic compartment, where dry biofilms can act as a nutrient resource for immigrant bacteria arriving there from terrestrial ecosystems. Since the subsuperficial compartments desiccate more slowly, and retain humidity for longer, the exposure to airborne bacteria is lower in the subsuperficial sediments. This difference was observed in the Fuirosos stream (Timoner et al., 2014a), where the epilithic bacterial community during the dry period was the most singular (less than 25% of OTUs shared with the other communities), whereas those inhabiting superficial (epipsammic) and subsuperficial (hyporheic) sands were highly similar to each other (62% of shared OTUs).

Less information is available for archaeal communities thriving in streambed habitats and their response to dryness. Studies addressing the effect of flow intermittency on archaeal communities are scarce compared to those with bacteria, and this can be taken as an indication that Archaea constitute a minor component of streambed microbial communities. Even in permanent rivers and streams Bacteria outnumber Archaea in all streambed compartments (Battin et al., 2001; Herrmann et al., 2011; Merbt et al., 2011, 2015; Buriánková et al., 2013), but is also true that Archaea occur in arid soils, microbial mats and freshwater sediments exposed to desiccation (Rothrock and (Rothrock and Garcia-Pichel, 2005; Soule et al., 2009; Timonen and Bomberg, 2009; Conrad et al., 2014). Despite this information, no conclusive data on the ecological function of the Archaea in streambeds are available. Some studies have identified either methanogenic groups (Buriánková et al., 2013; Mach et al., 2015) or molecular signatures of ammonia oxidizing archaea (AOA; Merbt et al., 2011, 2015) suggesting a relevant but not yet resolved contribution to C and N cycles, respectively. AOA are abundant in soils, where they play an outstanding role in nitrification (Stahl and de la Torre, 2012), but are less resistant to desiccation than ammonia oxidizing bacteria and less resilient to flow return, when ammonium concentrations are high (Thion and Prosser, 2014). Streambed habitats offer a wide array of anoxic micro-niches with low redox conditions, which potentially would allow the growth and activity of methanogenic archaea. The ability of some methanogenic species to survive oxygen exposure and desiccation stress in aerated soils (Angel et al., 2012) is an indication of their potential to occur in temporary streams even during the dry period. In fact, manipulative experiments in lake sediments have provided evidence that methane production persists during desiccation despite changes

in abundance and diversity of archaeal communities (Conrad et al., 2014).

The rewetting phase may be associated with a new bacterial community composition, as has been observed in some systems (Marxsen et al., 2010), but not so clearly in others (Febria et al., 2015). These contradictory results indicate that community changes may be particular for each benthic compartment, according to their particular exposure and resilience, and highlight the prevalent environmental conditions at each site as a factor determining the fate of the microbial communities. Timoner et al. (2014a) observed that biofilm on coarse sand, cobbles and hyporheic sediment reacted differently to the rewetting. OTUs affiliated to *Actinobacteria* and *Proteobacteria* were abundant in epipsammic and hyporheic biofilms, similarly to what is occurring in soils and river sediments (Gao et al., 2005; Tamames et al., 2010), while OTUs affiliated to the *Cyanobacteria* and the *Firmicutes* predominated on cobbles. These changes in community composition were accompanied by an increase in richness and diversity in epipsammic and hyporheic biofilms, likely favored by the increased number of niches provided by sand particles (Sigee, 2005).

FUNCTIONAL CHANGES AND ADAPTATIONS OF BIOFILMS TO DRY AND WET ALTERNATION

The photosynthetic efficiency, or the ability of the autotrophs to use their photosynthetic apparatus, is positively related to the percentage of water content (Timoner et al., 2012). As a result, a sharp decrease of the photosynthetic ability, even approaching zero during the terrestrial phase (Timoner et al., 2012), characterizes the response of autotrophs. These low values may remain until flow resumption, when the recovery response may differ between substrata, faster in the epilithic biofilm (the one having most of the chlorophyll), slower in the subsuperficial sediments (Timoner et al., 2012).

Water stress, high air temperatures, or direct effects of light are the factors mostly affecting chlorophyll degradation. Up to 60–90% of active chlorophyll (Chl-a) disappears during the streambed desiccation (Timoner et al., 2014b). However, this decrease in active chlorophyll comes along with a high proportion of accompanying pigments protecting the cell (**Table 1**). Two basic types of pigments are produced to protect algal tissues. Scytonemin is a colored pigment that occurs in the extracellular polysaccharide sheaths of cyanobacteria (Garcia-Pichel and Castenholz, 1991), and has been observed in microbial mats (Fernández-Valiente et al., 2007) and soil crust biofilms exposed to desiccation and high solar radiation (Belnap et al., 2005). Another group captures free radicals generated by UV penetration within the cell. These include echinenone, canthaxanthin, β-carotene, lutein, zeaxanthin, and myxoxanthophyll. These pigments occur intracellularly and protect cells from lethal singlet oxygen generated by UV or stress situations (Karsten et al., 1998). Some of these protective carotenoids were observed in Fuirosos (Timoner et al., 2014b). Echinenone was the first to occur, already by the end of the

TABLE 1 | Distribution of protective carotenoids associated to flow intermittency in the different algal groups from various sources (Rowan, 1989; Jeffrey et al., 1997; Buchaca, 2009; Timoner et al., 2014b).

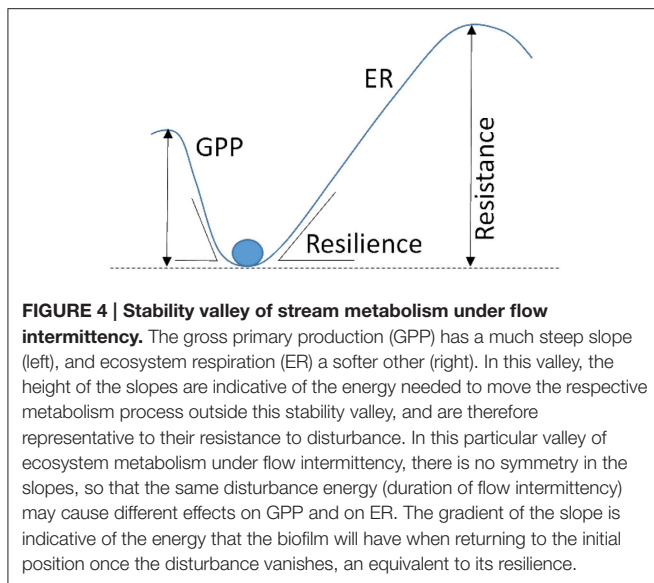|	Bacillariophyta (Diatoms)	Chlorophyta (Green-algae)	Cyanophyta (Cyanobacteria)	Cryptophyta	Chrysophyta (Yellow-green algae)	Euglenophyta Euglenophyta	Rhodophyta (Red algae)
EXTRACELLULAR							
Scytonemin			X				
INTRACELLULAR							
β, β –carotene	X	X	X	X	X	X	X
Cantaxanthin			X				
Echinenone			X				
Lutein		X					X
Myxoxanthophyll			X				
Zeaxanthin	X		X	X	X	X	X

drying phase, when the stream flow was extremely low, and consistently increased over the non-flow phase, and scytonemin followed on. The occurrence of these two carotenoids was evident also under low light availability, indicating that their synthesis was aimed to protect biofilm from desiccation and higher temperatures, not so much as a photoprotective mechanism. The occurrence of these carotenoids under stressful conditions may reflect an adaptive strategy to reduce long-term energy costs associated with cell repair (Flemming and Castenholz, 2007). In the Fuirosos, these physiological adjustments only occurred in the most exposed biofilm (the epilithic), allowing them to be more adaptive to desiccation stress and facilitate its physiological recovery immediately after rewetting.

Other roles of the bacterial and archaeal communities in the processing of organic matter and element cycling are poorly known, and our knowledge is even smaller on how they respond to flow intermittency. Some key ecosystem processes in intermittent streams, such as leaf litter decomposition, may be related to a rather unknown response of bacteria and fungi to the drying process. Rehydration events during non-flow (unpredictable rainfall events that do not imply runoff) produce activity pulses leading to high carbon degradation, momentarily alleviating microbial processes from moisture limitations (Timoner et al., 2014c). Similarly, enzyme activities and photosynthetic processes can be enhanced immediately after flow resumption to values higher than the ones observed during the aquatic period (Timoner et al., 2012). This enhanced activity resembles the "birch effect" (pulse of respiration on rewetting a dry soil), which may significantly influence the ecosystem carbon balance (Unger et al., 2010). This enhancement also occurs with nitrification and denitrification rates when flow resumes (Austin and Strauss, 2011; Gómez et al., 2012). Environmental changes occurring during the transition from wet to dry are a hot moment for microbial activity. For instance, oxygen depletion in pools isolated during river flow fragmentation because of the accumulated organic matter might favor the development of anaerobic microbial communities and the accumulation of reduced compounds such as sulfide and methane as a result of the activity of sulfate-reducing bacteria and methanogenic archaea, respectively (Briée et al., 2007).

THE IMPLICATIONS OF FLOW INTERMITTENCY FOR ECOSYSTEM METABOLISM

The spatial extent and the duration of non-flow events can vary from regular short dewatering and substratum drying of habitat patches, to prolonged reach-scale events (Stanley et al., 1997), and similarly happens with the duration of flow interruption. These differences in space and time may produce higher or lower effects on the community composition as well as on the physiological responses of both the autotrophic and heterotrophic components of biofilms. Given the key position of biofilms in the pathways of energy fluxes of freshwater ecosystems, these changes can be ultimately reflected in the ecosystem metabolism.

Flow intermittency does not only affect the total amount of organic matter available in freshwater ecosystems, but also the composition and quality of the available organic matter. Thus, the total polysaccharide, amino acid, and lipid content in the benthic organic matter may be higher during the drying process than in the recovery period (Ylla et al., 2010), while peptides may be minor. Summer, the period of the most common intermittency, shows the highest variability in organic matter acquisition, precisely when its quality is the most variable (Ylla et al., 2010; Romaní et al., 2013). In a study of the isolated pools in an intermittent stream, Fazi et al. (2013) showed that the degree of aerobic/anaerobic conditions was the main driver of the bacterial community composition, together with the typology of available DOM. Timoner et al. (2012) evaluated the activity of three different exoenzymatic activities during the drying- rewetting process, as potential estimates of the ability of the heterotrophs (and some autotrophs) to transform organic materials. They found that the microbial activities were related to the available materials but also were tightly associated to the water content in the different stream compartments. The extracellular enzyme activities generally decreased during the terrestrial phase, but the potential capacity of the bacterial community to degrade carbon (beta-glucosidase, BG) and phosphorus (alkaline phosphatase activity, AP) compounds remained, especially in the epipsammic and hyporheic biofilms. The low activity of the nitrogen-related enzyme (leucine-aminopeptidase activity, LAP) during this phase

FIGURE 4 | Stability valley of stream metabolism under flow intermittency. The gross primary production (GPP) has a much steep slope (left), and ecosystem respiration (ER) a softer other (right). In this valley, the height of the slopes are indicative of the energy needed to move the respective metabolism process outside this stability valley, and are therefore representative to their resistance to disturbance. In this particular valley of ecosystem metabolism under flow intermittency, there is no symmetry in the slopes, so that the same disturbance energy (duration of flow intermittency) may cause different effects on GPP and on ER. The gradient of the slope is indicative of the energy that the biofilm will have when returning to the initial position once the disturbance vanishes, an equivalent to its resilience.

indicated a potential restriction in the biofilm capacity to degrade peptides. These patterns indicated that bacteria preferentially used C and P during the terrestrial phase, most likely aiming to obtain energy rather than to obtain N for growth (Schimel et al., 2007). Flow return corresponds to a rapid increase of enzymatic activities, even after a prolonged dry period (Romani and Sabater, 1997). The biofilm material quality (i.e., the relationship between C and other elements such as N and P in the organism cells) changes according to these different activities in the different phases. Both the stoichiometric C:N and C:P ratios of epipsammic and hyporheic compartments increased with streambed desiccation (Timoner et al., 2012).

The first rains mobilize higher quality materials, when the DOC content reaches its maximum and the largest molecules are the most biodegradable (high BDOC values and high enzymatic activities; Romani et al., 2006). Large and rapid DOC inputs entering temporary streams during the transition to flow return provide available N and C sources for the heterotrophs that were previously (during the dry period) not available, and that the heterotrophs use efficiently. These changes between water phases indicate that several processes mediated by biofilms may remain compromised by the duration of the non-flow period. This duration might be understood as a key phase for temporary streams functioning.

All these changes in the quantity and quality of organic matter and nutrients ultimately influence ecosystem metabolism. Among the reported effects, the most remarkable is the uneven effect of exposure to non-flow conditions on autotrophic and heterotrophic processes; biofilm algae may be more affected than biofilm bacteria (Timoner et al., 2014b). This differential effect has also been reported at the meso-ecosystem scale, as respiration has been reported to be more resistant to flow intermittency than gross primary production (GPP; Acuña et al., 2015). Specifically,

Acuña et al. (2015) using a set of artificial channels with varying intensity of dryness, proved that the duration of the non-flow period differentially influenced autotrophic and heterotrophic processes, leading biofilms to a relative increase of heterotrophy. That study proved that autotrophic processes (such as primary production) were impaired at all durations of the non-flow events, while heterotrophs were able to persist under some degree of humidity, resulting in a non-linear respiration response. Overall, one might say that GPP is less resistant but more resilient than ecosystem respiration (ER), and that this asymmetry in the valley of ecosystem metabolism stability (**Figure 4**) results in different responses of each component to extreme flow events. In such a conceptual scheme, the GPP requires less energy to be moved outside its stability valley, while ER requires higher energy, and is therefore more resistant. Under flow temporality there is no symmetry in the slopes, so that the same energy for disturbance (duration of flow intermittency) may cause different effects on GPP and on ER. The gradient of the slope is also different for each of the two processes, defining an equivalent to its resilience, which is higher in the autotrophs and to the associated GPP of the ecosystem when flow returns.

FUTURE CHALLENGES

Research in temporary streams has been mostly performed up to now in temperate streams around the world, but many other temporary systems remain unexplored. River intermittency may occur naturally (e.g., as in Mediterranean rivers), or a result of unexpected events (e.g., El Niño influence), or stimulated by human activities. In these latter cases, the biofilm response might not coincide with that observed in the naturally- temporary systems, unless unexpected events or human practices occur regularly. The resistance and resilience responses shown by biofilms in Mediterranean and other temperate systems may not be repeated under these situations, but offer a template of the potential responses in those systems forced to temporality by global change.

AUTHOR CONTRIBUTIONS

SS organized the writing and assembled all the different contributions. VA contributed the part of biofilm implications for metabolism. XT contributed large part of the algae and pigment results. CB contributed a large part of the bacterial responses.

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