How increased dehydration duration affects the structure and functions of benthic biofilms

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Abstract

Global climate change has greatly increased the drought duration, frequency, and severity of intermittent river ephemeral stream (IRES), affecting the microbial-mediated biogeochemical process. While there is limited information about the responses of community structure and ecosystem functions of benthic biofilms in IRES, especially under the increased drought duration. Here, we focused on the increased drought duration and summarized their effects on the structure and functions of benthic biofilms in IRES. First, the increased dehydration duration led to distinct effects on the α-diversity or β-diversity of benthic microbial communities. The interaction network should be considered in future research as they are essential to maintain biofilm structure and play key roles in the resistance and resilience in biofilm community recovery under hydrological stress. In addition, inconsistent response patterns of the fundamental functions, such as gross primary production, ecosystem respiration, and functional enzymes activity of biofilms were discussed. Besides, the emissions of greenhouse gases (GHGs) of biofilms in IRES deserve more attention due to that their emission flux of biofilms could be significantly altered after prolong dehydration duration with a huge pulse when rewetting. More important, it is ecosystem multifunctionality rather than a single function that needs to be fully considered when studying the microbial functions and the biogeochemical process mediated by biofilms in IRES under increased dehydration duration. Also, more research is needed at larger spatial and longer temporal scales to evaluate the effects from a more macro perspective for better understanding the ecological impacts of increased dehydration duration in IRES ecosystems.

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Key Points:

- The interaction network should be considered in future research, which play key roles in the biofilm community resistance and resilience.
- The emission of biofilms greenhouse gases may be significantly altered after prolong drought duration with a huge pulse with rewetting.
- Ecosystem multifunctionality needs to be fully considered when studying the biogeochemical process mediated by biofilms in IRES.

Abstract:

Global climate change has greatly increased the drought duration, frequency, and severity of intermittent river ephemeral stream (IRES), affecting the microbial-mediated biogeochemical process. While there is limited information about the responses of community structure and ecosystem functions of benthic biofilms in IRES, especially under the increased drought duration. Here, we focused on the increased drought duration and summarized their effects on the structure and functions of benthic biofilms in IRES. First, the increased dehydration duration led to distinct effects on the α-diversity or β-diversity of benthic microbial communities. The interaction network should be considered in future research as they are essential to maintain biofilm structure and play key roles in the resistance and resilience in biofilm community recovery under hydrological stress. In addition, inconsistent response patterns of the fundamental functions, such as gross primary production, ecosystem respiration, and functional enzymes activity of biofilms were discussed. Besides, the emissions of greenhouse gases (GHGs) of biofilms in IRES deserve more attention due to that their emission flux of biofilms could be significantly altered after prolong dehydration duration with a huge pulse when rewetting. More important, it is ecosystem multifunctionality rather than a single function that needs to be fully considered when studying the microbial functions and the biogeochemical process mediated by biofilms in IRES under increased dehydration duration. Also, more research is needed at larger spatial and longer temporal scales to evaluate the effects from a more macro perspective for better understanding the ecological impacts of increased dehydration duration in IRES ecosystems.

Keywords: Dehydration duration; Biofilm; Ecosystem multifunctionality; Emissions of greenhouse gases

Content

1. Introduction

Flowing rivers and streams provide important resources for human beings and support multiple ecosystems by transporting sediment, organic material and nutrients (Jaeger 2021). For now, 51–60% of global rivers and streams are intermittent (hereafter is referred collectively as the intermittent rivers and ephemeral streams or IRES) (Mathis, et al. 2021), and owing to the limitation to quantifying and mapping IRES at global scales, these numbers of IRES have been probably underestimated, indicating that such temporal dynamism is increasingly prevalent (Acuña, et al. 2014). IRES are recognized as biogeochemical reactors, metabolizing organic carbon with net emission of greenhouse gases (GHG) to the atmosphere (Battin, et al. 2023). Moreover, with the decreases in regional precipitation and global warming, drought becomes more frequent and severer (Sheffield, et al. 2012), resulting in further changes in the global distribution and the extent of IRES (Arias-Real, et al. 2020). The increased IRES will affect how the biodiversity of ecological communities varies in both space and time scales (Sarremejane, et al. 2020). Even if the flow recovers, it is difficult to determine whether the original state comes back together; thus, impacts on the ecosystem can be profound and lasting. Therefore, our understanding of flow intermittency can't stay at the straightforward dry-wet cycles because if flow ceases again before full recovery, a new state may replace the previous ecosystem (Schwalm, et al. 2017). After increased dehydration duration, what the new state will be and why the ecosystem goes for it are questions to be answered.

To answer these, we first sought to figure out how increased dehydration duration affects community structure and ecosystem multifunction. Accordingly, fluctuating flow, especially intermittence, is the most leading factor mediating microbial functioning in IRES (Romaní, et al. 2017). Flow intermittency triggers a chain of cascading effects, influencing the biodiversity, community structure and microbial functions (Sergi Sabater 2016). To a large extent, the spatial and temporal distribution of benthic microbial communities in IRES, their resistance and resilience capacities affect interaction network and ecosystem multifunctionality (Romaní, et al. 2017). Even though studies have already characterized the effects of non-flow periods on stream ecosystems, the resistance and resilience mechanisms and ecosystem multifunctionality of biofilms in IRES remain poorly understood. In addition, ecosystem restoration in IRES requires multi-dimensional consideration (Moreno-Mateos, et al. 2020), such as comprehensive research on ecosystem structure, function and stability. In a nutshell, the far-reaching influence of increased dehydration duration ought to be taken into account at global scales and the mechanism of biofilm responses to the dynamic changes needs light to be shed on at more comprehensive scales.

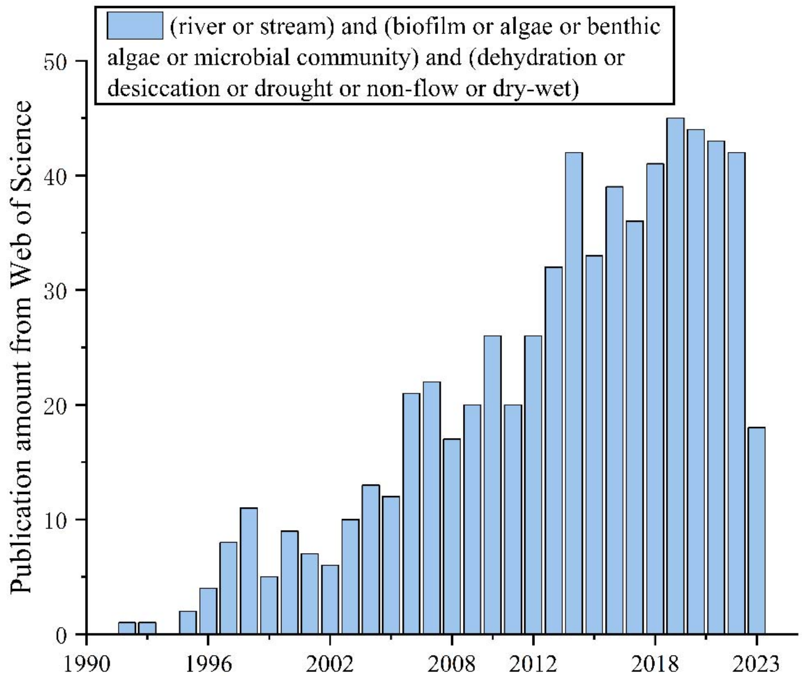

Figure 1. The number of publications from Web of Science (from 1992 to 2023, updated to April, 2023). The data collection was conducted based on key words of the research of biofilms in IRES as well as dehydration.

2. Intermittent rivers and biofilms

IRES are experiencing alternating wet and dry phases where the drying usually operates in longitudinal and vertical dimensions (Sergi Sabater 2016), causing temporal fluctuations in aquatic biodiversity. First, as basal flow decreases, flow interruption proceeds steadily, and evaporation and infiltration of superficial waters cause shallow surface habitats to disappear. In this case, pools may persist with a low water flow in the hyporheic zone, namely superficial dryness. Then, the deep dry phase comes, and the phreatic layer can descend, causing the complete hydrological isolation of the river compartments. When rainfall comes or the flow return, the dry riverbed begins rewetting, which means the recovery can be uncertain and may occur only after a wet period rather than a given duration after a drought (Peterson, et al. 2021). Due to climate change and anthropogenic pressures, IRES is becoming more common globally, which puts forward higher requirements to understand and manage the severe degradation of aquatic ecosystems and its adverse impacts on our societies (Acuña, et al. 2014). Increases in drought frequency, severity and duration triggered poor water quality conditions in boreal streams, thus could even change the functional role of rivers and streams worldwide (Lluís, et al. 2020). Here, we primarily focus on increased dehydration duration and probe into how it influences IRES ecosystem.

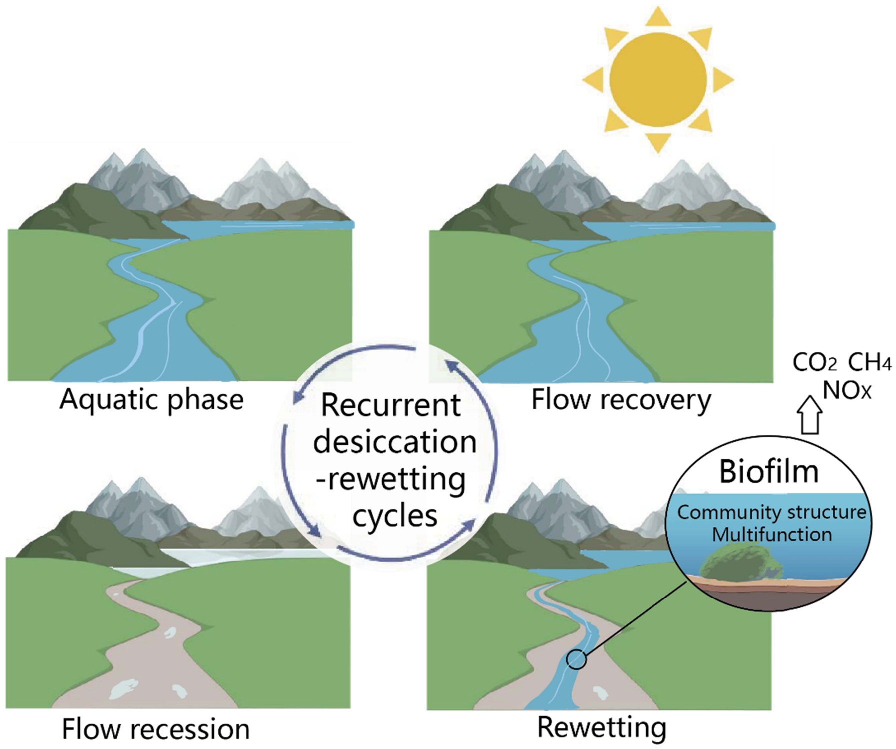

Figure 2. Recurrent desiccation-rewetting cycles in IRES. Four phases exist in the cycles of IRES: aquatic phase, flow recession, flow recovery, and rewetting. Increased dehydration duration can impact both community structure and the multifunctional biofilm living in IRES, thus substantially affecting greenhouse gas fluxes.

Biofilms are a complex and functional collection of microorganisms composed of microorganisms and their secreted extracellular polymers (Battin, et al. 2016), including 10% microorganisms and 90% extracellular polymeric substances (EPS) that constitute. As the main biological interface in the river ecosystem (Miriam, et al. 2019), biofilms are crucial in driving the river ecosystem's primary production, material circulation and energy flow. At the same time, they are also recognized as substantial contributors to greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (NOx) emissions into the atmosphere (Battin, et al. 2023). Stream benthic biofilms alter their structural function and community composition during different hydrological phases. Therefore, biofilms, an indicator of ecosystem structure and function, have gradually gained attention in the study of IRES.

Microbial communities in biofilms are critical to adapting to the environment and recovering and diffusing after rehydration. The possibility lies in that any place that provides a temporary moist environment can become a living place for biofilms. However, the survival of microbes still depends on the duration of the drought: in systems with long dry times and harsh conditions, most microbes will not survive. The watershed runoff cannot recover as expected, and the ecosystem's biodiversity and ecological functions also show similar responses, which has brought significant challenges to their research and management. The biofilm function has resistance and resilience to drought, but this capacity is not infinite, and the recovery mechanism is still in suspense (Peterson, et al. 2021). Therefore, further research needs to extend the current knowledge about biofilms' structural and functional responses and the resistance mechanism during increased dehydration duration in IRES.

3. The adaptive changes of biofilm structure in response to dry-wet stress

3.1 The changes in microbial composition and community structure

During the flow recession process in IRES, pools were formed in the deeper part. Since dryness is not homogeneous, patches may occur along the riverbanks, with reef and rock land partitions providing more niches, creating higher than expected Beta diversity (Elisabet, et al. 2021). The native communities were gradually replaced by other aquatic, semi-aquatic or terrestrial communities. In addition, the increased dehydration duration determines the biofilm's structural response and leads to the transition of the biofilm structure. Prolonged droughts hinder biofilm resistance structure formation; thus, the community structure may have difficulty returning to its original state.

Table 1. Research progress of the microbial composition and community structure. The journals used in this survey have been published in recent five years in which researchers studied biofilms in IRES worldwide. They pointed out how increased dehydration duration influences the microbial composition and community structure.

o	**Research object**	**Period**	**Sites**	**Methods**	**Reference**
	Algal communities	51 days	Streams in northeast of the Iberian Peninsula	In artificial streams	Timoner, et al. 2019
	Benthic algae communities	1 year	five lowland streams around Aarhus in Denmark	Sampling and measuring	Kun, et al. 2020
	Benthic diatoms	12 weeks	Streams in southern Portugal	Sampling and measuring	Novais, et al. 2020
	Benthic algae communities	4 weeks	In 12 outdoor flumes in Denmark	Sampling and measuring	Annette Baattrup-Pedersena 2020
	the structure and diversity of stream benthic diatoms	2 months	23 Mediterranean temporary streams	Sampling and measuring	Elisabet, et al. 2021)
	Density and biomass of agal and bacterial communities	/	/	review	Sergi Sabater 2016
	Microbial decomposition and fungal biomass	365 days	20 streams across Catalonia (Spain)	Sampling and measuring	Arias-Real, et al. 2020
	Fungal biodiversity and ecosystem processes	1 year	15 low-order Streams across Catalonia	Observational and manipulative experiments	Arias-Real, et al. 2021
	Bacterial communities	/	/	review	Romaní, et al. 2017
0	streambed microbial density, diversity, composition	8 months	Mediterranean intermittent streams	Sampling and measuring	Giulia, et al. 2020
1	Fungi and bacteria diversity and microbiome complexity	/	In soil	feature selection	Wagg, et al. 2019
2	Fungi and bacteria decomposers	/	in two streams with distinct characteristics	*in situ* experiment	Duarte, et al. 2017
3	Microbial Compositions	35 days	In lab	In lab	Miao, et al. 2021
4	Microbial decomposition and fungal biomass	one hydrological year	20 streams across Catalonia	Sampling and measuring	Arias-Real, et al. 2020
5	Bacterial density and basal fluorescence	60 days	Sixteen artificial streams	Sampling and measuring	Muñoz, et al. 2018

3.1.1 Algae and bacteria in biofilms

The community structure and composition of biofilms as the biological consortia, has recently gained some traction. The responses of the main biological components of biofilms——algae cyanobacteria, bacteria, and fungi to increased dehydration duration have been reviewed by (Sergi, et al. 2016) from the point of view of microbial physiology. Studies found there were no significant differences between different aquatic regimes detected for benthic diatom species richness, while Shannon diversity and Pielou's Evenness indices showed differences (Novais, et al. 2020). It indicated that the changes induced by flow intermittency were distinct in α-diversity rather than similar species richness. However, research demonstrated no distinct differences in diatom α-diversity, whilst differences in beta diversity between intermittent and permanent streams (Elisabet, et al. 2021). The inconsistency of their conclusions possibly resulted from the climate characteristics of the Mediterranean area, causing the minimum differences between temporary and permanent streams. Furthermore, a study found in 150 days duration of non-flow period, α-diversity and evenness of benthic diatoms both showed an increase in the first 50 days and finally decline in the third (Elisabet, et al. 2021), which implies the trend of community composition and structure as well as the possible threshold during the dry days. These exciting findings above encouraged further research, from external changes in community structure to the mysterious mechanisms inside.

The response patterns of bacteria and algae to hydrological stress are proven to be different. (Sergi, et al. 2016) reviewed that both bacteria and algae experience significant decreases in cell densities and biomass, while the former is influenced slower, indicating that heterotrophs are more resistant to dry and wet stress than autotrophs. Increased dryness tended to modify the bacterial communities' composition and reduce α-diversity. Some kinds of bacteria were observed to temporarily replace the others in biofilms during the non-flow period in a temporary stream, such as Actinobacteria and Firmicutes replaced Cyanobacteria. Those bacteria might be more dominant in IRES since these phyla have several rRNA operons in the genomes, which is considered key to competitive success during periods of maximum dehydration (Romaní, et al. 2017). In contrast to community composition, richness and diversity were not significantly modified by hydrological history, suggesting that there was taxa-dominance alternation or substitution without increased dryness significantly affecting the overall diversity (Gionchetta, et al. 2020). Furthermore, different microbial species are sensitive to dryness at different spatial scales. The specific sensitivity of each population to the hydrological conditions determines waves of microbial functions and covariation of biodiversity, which can determine ecosystem biodiversity and functioning (Duarte, et al. 2017; Gionchetta, et al. 2020). Recent studies found that different environmental conditions, such as artificial substrates can make the fate of microbial compositions and interactions different, likely favored by the special niches for microbial colonization (Miao, et al. 2021), which encourages further study of microbial aggregations, interactions, and functions.

3.1.2 Fungi and archaea in biofilms

Currently, most studies focus on the community structure, abundance and diversity of algae and bacteria in biofilms. In contrast, the response of fungi and archaea in biofilms to hydric stress is rarely studied. Based on the related studies about soil drought, we speculate that fungi and archaea showed special differences compared to bacteria, leading to great significance to community dynamics. Recent studies highlighted the research value of fungi and archaea in the river ecosystem, especially their response mechanism and contribution to C and N cycle. In addition, archaea were previously thought to be only a minor component of biofilm microbial communities. However, current studies have shown that methanogenic groups or ammonia-oxidizing archaea play an important role in C and N cycles. Some methanogenic species can survive dehydrations, suggesting they may also exist in intermittent flows during drought. Indeed, archaeal activity and methane production persist during dehydration despite changes in the number and diversity of archaeal communities (Arias-Real, et al. 2020).

Fungi play an important role in decomposing submerged organic matter (Besemer 2015). It is expected that fungal communities in IRES have traits that better adapt to IRES environmental conditions, such as the potential adaptation of the aquatic hyphomycetes' amphibious nature (Romaní, et al. 2017). Fungal richness positively affected organic matter decomposition and fungal biomass accrual (Arias-Real, et al. 2021). On the other hand, fungi are sensitive to drought but are more resistant than prokaryotes (Duarte, et al. 2017). Nevertheless, except during droughts, the decisive factor is probably the lack of oxygen rather than the water for aquatic fungi (Bärlocher and Boddy 2016). Heino also found a striking difference in the community–environment relationships between fungi and bacteria (Heino, et al. 2014), indicating their special response to hydric stress in biofilms. Despite the general understanding, the role of fungi is still poorly understood compared to bacteria in biofilms during hydric stress (Gionchetta, et al. 2020). Moreover, there is limited information regarding the responses of microeukaryotic communities such as fungi and metazoan under increased dehydration duration (Miao, et al. 2021). Much of the variation in fungal and archaeal communities remained unexplained, suggesting that drivers of these communities are likely to be complex and not yet fully clear (Heino, et al. 2014). Each component of biofilm communities is indispensable because of the specific functions they perform in the ecosystem. Thus, lacking research, anyone is insufficient to fully reflect the structure and multifunctionality of biofilms.

3.2 The changes in interaction network within biofilm communities

Accordingly, multispecies in biofilms exhibit complicated microbial communication, interaction, and cooperation, and the microbial complexity, particularly ecological networks, acts as more complex attributes than alpha- and beta-diversity patterns, providing further information about microbial interaction in biofilms (Miao, et al. 2021). Communities' cooperation and interaction network are essential to forming biofilm structure and thus can't be ignored. Recently, co-occurrence network analysis has been tried to explore the symbiotic patterns in complex microbial communities and their responses to environmental changes (Miao, et al. 2021). Bacterial co-occurrence networks were found stronger, larger, more constant, more connected and less modular than fungal networks, especially the response to drought. The stability of the former was weakened by drought, and their response was more closely related to soil function than the latter during soil moisture recovery (de Vries, et al. 2018). Liu, et al. (2020) also showed no significant difference in α-diversity indices of bacterial and eukaryotic communities, while a reverse trend was found in Shannon indices. They both implied that remarkable differences appeared in bacteria and fungi when responding to environmental changes, particularly the co-occurrence network structure. Thus, increasing evidence shows that ecological networks can influence communities' responses to climate extremes such as drought.

There is a need to extend community analyses beyond exploring species richness and community structure (Barberan, et al. 2012). Owing to the great significance of maintaining the functions of ecosystems, more research is needed to explore mechanisms of interaction networks within biofilms in intermittent rivers. Despite the common underlying ecological dynamics, the fungi differ from the bacteria communities regarding temporal structure, response to drought and heritability at different compartments (Gao, et al. 2020). Additionally, in large rivers, fungi have a stronger dispersal limitation influence and less network connectivity than bacteria, indicating different community assembly mechanisms and ecological functions between the two (Chen, et al. 2020). Regarding biofilms under dry-wet stress, we proposed a hypothesis that the difference in community structure will lead to that in ecological function, which has yet to be well known nowadays.

Seminal studies suggested that enhancing network complexity could improve the stability and maintain the biodiversity of microbial communities. For instance, research established the ecological network helping to understand the structure of complex microbial communities and spatio-temporal dynamics of soil microbial communities (Barberan, et al. 2012). In addition, Coyte, et al. (2015) found the correlations between mechanisms for maintaining stability and cooperating networks, enlightening us about the possible relationship between ecological stability and biofilm diversity. The network stability of different groups is significantly different, and the stability of the ecosystem is closely related to the network (de Vries, et al. 2018). Given such a close correlation, the reconstruction and restoration of the entire ecological network should be considered in ecosystem restoration rather than certain communities or diversity. Consequently, we should consider the specific effects of cooperation and how cooperation interacts with other community characteristics, which are significant to community dynamics. In addition, from a long-term perspective, the analysis methods of biological interaction networks and evolutionary potential should be integrated to repair the complexity of degraded ecosystems (Moreno-Mateos, et al. 2020). To better understand the biofilm-formation pattern and potential microbial interaction in intermittent rivers, it is necessary to employ co-occurrence network analysis on biofilm and explore the differentiation of network patterns under dry-wet stress. Communities' cooperation and interaction network are essential to forming biofilm structure and thus can't be ignored. When employing the co-occurrence network analysis, more and further diversity indexes besides α-diversity or β-diversity to understand the complex structure of microbial communities in future research.

3.3 Resistance and resilience mechanisms in biofilm community recovery

Dehydration and the dry riverbed in IRES will choose which communities to survive. Because of the cooperative relationship between the communities, the community's resistance and resilience recover. Those organisms that exist until rewetting and recovery are called “resistant” and “resilient” to the effects of flow intermittency (Bogan, et al. 2017). To better understand biofilm recovery in IRES, more than knowledge of community structure and co-occurrence is required. Increased dehydration duration reduced fungal species richness and caused compositional changes driven by species turnover, suggesting resistance mechanisms to cope with drying (Romaní, et al. 2017). As microbial communities are complex and flexible to environmental changes, further study is needed to examine their resistance and resilience to hydrological stress mechanisms.

Here, we define resistance as the capacity of biofilms to withstand drying. Resilience is recognized as the ability of biofilms exposed to dehydration-rewetting to recover rapidly (Zlatanovic, et al. 2018) and revert to ecosystem functions before dehydration duration or a new state. Biofilms display resistance regarding their ability to withstand a disturbance and resilience to recover following disturbances (Bogan, et al. 2017). Their intrinsic properties and extrinsic condition influence resistance and resilience (Shen, et al. 2022). Studies in soil found that different community components result in different responses to drought, with fungi being generally more resistant but less resilient than bacteria (de Vries, et al. 2018). Thus, we can conclude that there are similar phenomena in IRES. Additionally, bacterial networks on artificial substrates were more complex than those on natural substrates, implying that the former condition stimulates stronger stability and resistance of biofilm to external interference (Miao, et al. 2021). Studies found biofilms from permanent streams were less resistant to drying than those from temporary streams at structural level, but responded similarly at a functional level (Timoner, et al. 2019). Nonetheless, the benthic algae community structure and biofilm metabolism displayed similar resilience to the stress imposed by low flow in combination with co-occurring stress from nutrients and sediments on a short and long time scale (Annette Baattrup-Pedersena 2020). Hence, resistance and resilience of biofilm are related to increased dehydration duration and show complex relevance with the community structure and functions.

A previous study suggested that the contributions of resistance and resilience likely support the long-term stability and persistence of communities in IRES (Catherine Leigh 2016), while nowadays, it is necessary to better understand the intrinsic mechanisms of biofilms during t dry-wet stress. Resistance and resilience are essential in ecological research and the basis for threshold determination. Even if the intensity of human activities is higher than the average level of the global coastal ecosystem, the overall microbial community can recover to a state similar to the original state in a short time after the stress factors of the large-scale ecosystem are removed (Huang, et al. 2020). It demonstrated that the resistance and resilience of microbial communities are essential for ecosystem stability. Biodiversity is one important aspect of enhancing resilience, stabilising ecosystem productivity and related ecosystem functions by increasing resilience to adverse climates (Isbell, et al. 2015). Moreover, Gao found that compared with bacteria, fungi are more resistant to dehydration stress, but less resilient when rewetting to alleviate stress (Gao, et al. 2022). Thus, it is feasible to determine the maximum environmental conditions that the ecosystem can accept through the responses of the biofilm to disturbances. Researchers studied the impacts of the total duration and severity of the non-flow period by monitoring biofilm biomass and metabolism in IRES (Miriam, et al. 2019). The study also found that the taxonomical and functional composition of diatom assemblages mostly responded to the increased duration of the non-flow period, irrespective of whether these were consecutive (Elisabet, et al. 2021). More gradient designs in ecological experiments could be a major step toward studying the response mechanism to continuous and interacting environmental changes in a feasible and statistically robust way (Kreyling, et al. 2018). Therefore, the next step is furthering the gradient setting and analyzing the threshold range to provide scientific support for analyzing and protecting the ecosystem.

4. Ecosystem functionality of biofilms in intermittent rivers

4.1 Fundamental functionality of biofilms

Biofilm is a central contributor to primary production, nutrient cycling, and organic matter biodegradation, with potential implications for ecosystem functioning (Ferran, et al. 2020). Therefore, it is particularly significant to explore the functional characteristics of microbial communities (Miao, et al. 2021). Fundamental functions, such as gross primary production (GPP), ecosystem respiration (ER), autotrophic index (AI), biomass and functional enzymes activity in carbon (C), nitrogen (N), and phosphorus (P) cycles, are the foundation of the biofilm ecosystem. However, they are constrained during non-flow periods when affected by water stress, higher temperatures, and stronger solar irradiance (Timoner, et al. 2019). Many researchers have studied them, and previous findings showed that biofilm functions respond more strongly to hydrological stress than density and diversity do (Giulia, et al. 2020).

Responses of nutrient cycling and organic matter biodegradation in biofilms are complex. Dehydration reduced whole system metabolism, suppressing the rates of GPP and ER, but elevated dissolved organic carbon (Raquel, et al. 2021). Analyzing from headwaters to the main stem of the Connecticut River, Hosen concluded that in larger rivers, GPP increased more than ER during dehydration, even leading to temporary autotrophy (Hosen, et al. 2019). Despite the negative effect of high flow events and flow variations on biomass and biofilm community functions, positive effects on function-biomass ratios were also observed (Kun, et al. 2020). As for functional enzymes activity in N, P cycles, dehydration decreased the activities of the extracellular enzymes alkaline phosphatase, leucine aminopeptidase, phenol oxidase and phenol peroxidase, whereas it positively affected β-xylosidase activity (Schreckinger, et al. 2021). However, (Romaní, et al. 2017) found that during drying, peptidase activity revealed heavy use of organic-N compounds in stream biofilms while phosphatase activity remained in the sediment (Romaní, et al. 2017). Most enzymes declined by 30–80% during dehydration, whereas phosphatase activity increased by 60% after four weeks of fast dehydration. Upon rewetting, the activities of all enzymes increased above the initial wet state and tended to over-compensate for their dehydration losses so that processes such as denitrification and nitrification are quickly reactivated after recovery (Wesley, et al. 2021). During recovery, phosphatase and beta-xylosidase approached their initial wet state, while alpha-glucosidase increased continuously in the four weeks (Pohlon, et al. 2018). Different patterns of dehydration mode and time also affect nutrient cycling. Research found in sediment from the Breitenbach that activity of alpha-glucosidase increased more after a shorter dehydration time, while aminopeptidase activity increased after longer dehydration (Pohlon, et al. 2018).

Table 2. Research progress of the dynamic changes of biofilm function in IRES. This table displays responses of functional indicators to dehydration, implying dynamic changes in biofilm function in IRES worldwide.

No	Functional indicators	Main Findings	Methods	Periods	Sites	Reference
1	GPP, CR, total biofilm biomass	Longer durations of the non-flow period or high severity conditions might decrease GPP promoting heterotrophy	*In situ* Sampling and measuring	July 1st–August 1st	Mediterranean streams in NE Iberian Peninsula	Miriam, et al. 2019
2	GPP, CR	Benthic algae community and biofilm metabolism displayed similar resilience to low flow stress and co-occurring stress from nutrients and sediments	Semi-experimental approach	4 weeks in summer	12 outdoor flumes in Denmark	Annette Baattrup-Pedersena 2020
3	Biofilm metabolism and nutrient uptake	Including short-period hydrological conditions in studies significantly influences environmental factors shaping benthic algae.	*In situ* Sampling and measuring	More than 1 year	5 lowland streams around Aarhus in Denmark	Kun, et al. 2020
4	OM decomposition	Upon rewetting, extracellular enzyme activities in IRES are quickly recovered and the use of polysaccharides is enhanced.	review	/	/	Romaní, et al. 2017
5	C cycling	At longer dehydration, CH_4 dropped abruptly, CO_2 fluxes ceased later. Rainfall boosted fluxes of CO_2.	*In situ* Sampling and measuring	9 weeks	Lowland stream in Brandenburg	Arce, et al. 2021
6	extracellular enzyme activities and respiration	Microbial functions respond more strongly to hydrological factors than bacterial density and diversity do	Sampling and measuring	8 months	Mediterranean intermittent streams	Giulia, et al. 2020
7	GPP and ER	In larger rivers, GPP increased more than ER during dehydration, even leading to temporary autotrophy.	*In situ* Sampling and measuring	2015-2017	The Connecticut River	Hosen, et al. 2019
8	GPP, ER and DOC	Low flow reduced whole system metabolism, suppressing the rates of GPP	Stream mesocosm experiment	2 months	24 outdoor streams	Raquel, et al. 2021

		and ER, but elevated DOC concentration.				
9	CR and extracellular enzymatic activities	Milder drying surprisingly triggered a more rapid and drastic change in the microbial community composition and diversity	*In situ* Sampling and measuring	90 days	The Spree river in German	Schreckinger, et al. 2021
10	Carbon cycle	Organic carbon is mineralized and released mostly by microbial community to the atmosphere as CO_2 or CH_4.	*In situ* Sampling and measuring	/	More than 1000 river and riparian sites	Scott, et al. 2019
11	N cycle	An increase of N_2O emissions occurred at early drying before substantially dropping until the end of the experiment.	*In situ* Sampling and measuring	9-week dry period	A 100-m reach of Fredersdorfer	Arce, et al. 2018
12	extracellular enzymes	The activity of alpha-glucosidase increased more after a shorter dehydration time, but aminopeptidase activity after longer dehydration.	*In situ* Sampling and measuring	6 months	A first-order Central European upland stream	Pohlon, et al. 2018
13	extracellular enzyme activities, EPS	the EPS matrix is an efficient and flexible protection for attached bacteria against dehydration, pH stress, and various other stressors	*In situ* Sampling and measuring	/	20 headwater streams in Austria	Coulson, et al. 2022
14	microbial interactions, and functions	The bacterial communities on artificial substrates had stronger stability and resistance to external interference.	Laboratory incubation	35 days	In lab	Miao, et al. 2021
15	N transformation	Nitrogen transformation processes (e.g., denitrification and nitrification) are quickly reactivated after rewetting.	Laboratory incubation	Sep.4 to Dec.13 in 2019	Eighteen indoor artificial streams	Wesley, et al. 2021

Above conclusions indicated that the responses of nutrient cycling, and organic matter biodegradation and recovery mechanisms in biofilms are complex. The response of CO2 production activities to drying was advanced compared to that of extracellular enzymatic (Schreckinger, et al. 2021). Researchers also argued that bacteria preferentially use carbon and phosphorus during the dehydration phase, most likely to gain energy rather than to grow, implying the delicate mechanism of reacting to external interference in the microbial community (Scott, et al. 2019). Furthermore, compared to the macro-external changes, recent study indicated that intrinsic properties inside the IRES can also significantly influence the recovery of biofilms, even more than moisture content and history of intermittency. (Coulson, et al. 2022) highlighted that the effects of both active intermittent flow and history of flow intermittency can be overlaid by numerous other factors, such as sediment characteristics, elevated nutrient levels, temperature, and seasonality. Especially in temperate-climate zones, where intermittent flow conditions are often less harsh than in dry regions, stream and catchment characteristics may influence biofilm structures and processes stronger than intermittent flow conditions and compensate for or amplify dehydration effects (Coulson, et al. 2022). Moreover, different functions may show distinct variations during long-term dehydration, so some may recover while others cannot (Figure 3). Different function indexes such as AI, ecosystem metabolism and carbon metabolism show different responses to short-term and long-term dehydration. Our recent research proved that both AI and ecosystem metabolism can almost return to the original state either after short-term or long-term dehydration. In contrast, certain functions such as carbon metabolism cannot recover after long-term dehydration (Miao, et al. 2023). Therefore, as dehydration duration increases, certain ecosystem functions have a possibility of no recovery. Both the possibility and those functions are the key factor to determine the ecosystem threshold value, worthing attention and further research.

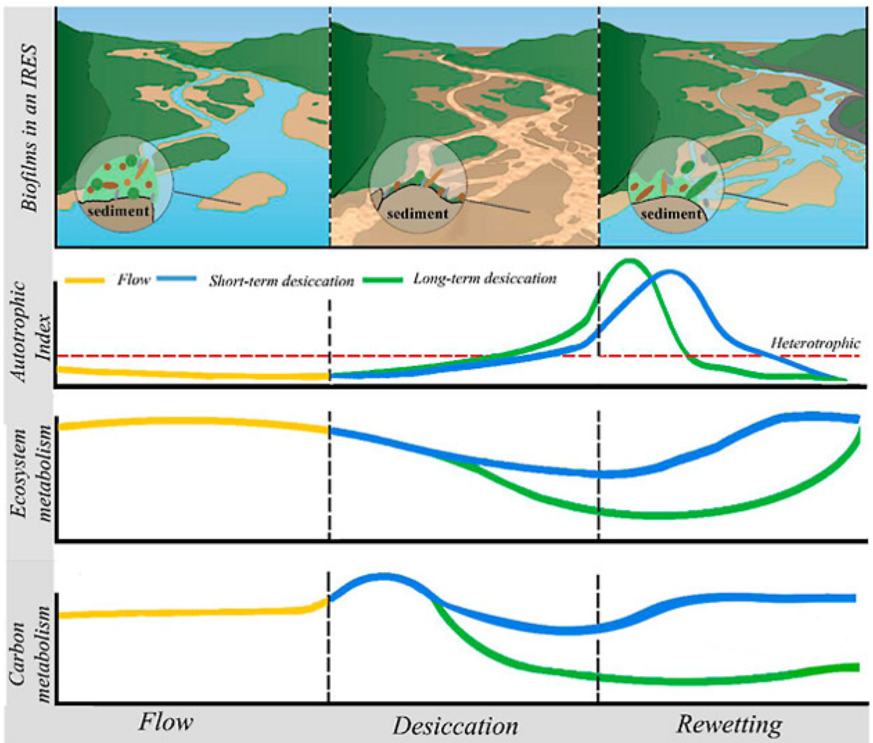

Figure 3. Dynamic changes of metabolic functions of biofilms, including AI, ecosystem metabolism and carbon metabolism in IRES drying and rewetting period (Miao, et al. 2023). Different indexes showed distinct responses to short-term and long-term dehydration.

4.2 The emission of greenhouse gases

Apart from these fundamental functions investigated, some special characteristics of biofilm functions during increased dehydration duration are understudied. Evidence shows that IRES's contribution to respiration and GHG emissions can be largely underestimated. Organic carbon is mineralized and released mostly by microbial communities to the atmosphere as CO_2 or CH_4 (Scott, et al. 2019). Given that different gases may show different variations during dry-wet alternations (Kosten, et al. 2018), this review, based on previous studies, summarizes the dynamic changes of GHG fluxes during dry-wet alternations using carbon dioxide flux as a typical example (Figure 4). When dehydration begins, researchers found that carbon metabolism and CO_2 flux decline, resulting from the microbial community composition and structure changes (Kosten, et al. 2018). Study reported twice the CO_2 release flux during the non-flow period than that in the normal flow (Gómez-Gener, et al. 2016). Especially after the dry riverbeds appeared, large amounts of CO2 and N2O were released from the sediment exposed to the atmospheric environment (Kosten, et al. 2018; Pinto, et al. 2022). Examining sediments from 200 dry IRES reaches spanning multiple biomes, researchers found mean respiration increased 32-fold to 66-fold upon sediment rewetting (Schiller, et al. 2019). At longer dehydration, CO2 flux declined again, likely because of reduced abundance of anaerobic microbial traits (Arce, et al. 2021). During rewetting, the communities may partially recover through water infiltration, making a CO2 flux pulse (Schiller, et al. 2019). Research found that carbon metabolism may recover under short-term dehydration, while it may be damaged under long-term and cannot return even if the flow returns (Miao, et al. 2023; Schreckinger, et al. 2022). After the flow recovery, a new state may take the place of the original one, while whether the GHG flux recovers or not is to be confirmed by future research (Miao, et al. 2023).

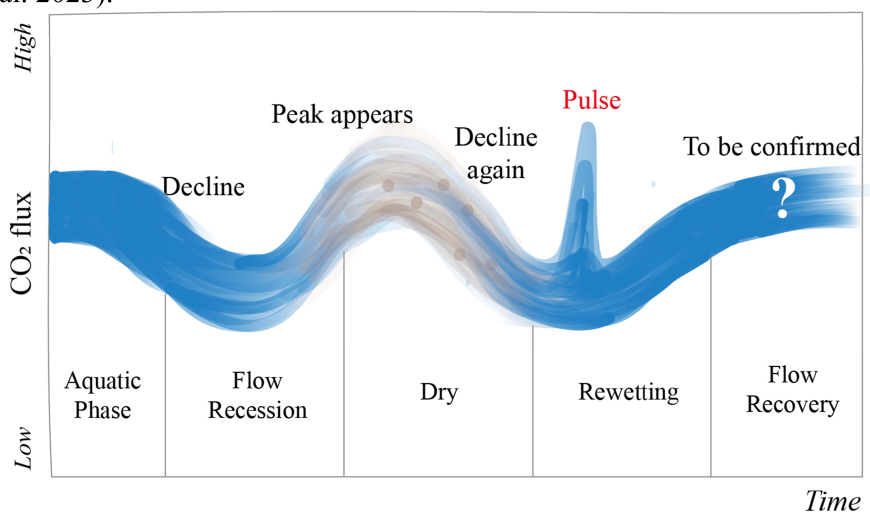

Figure 4. Dynamic changes of CO_2 fluxes in IRES drying and rewetting period. This diagram shows predictive CO_2 fluxes by IRES during different aquatic periods. When dehydration comes, metabolic functions begin to decline as well as GHG fluxes. During dryness, a peak may appear. After that, flow recovery may bring a CO_2 pulse which shows much higher flux and flow recovery may make a new state rather than the original one.

Owing to the capacity to accumulate large amounts of organic matter during the dry phase, rewetting events in turn may accelerate carbon processing in IRES (Rubén, et al. 2021). (Giulia, et al. 2020) also found in Mediterranean IRES that microbial functional metrics revealed a progressive increase in recalcitrant carbon degradation activity at sites with an extended dry phase. Furthermore, biofilms may become more heterotrophic with climate change, resulting in faster processing of recalcitrant carbon. Further research is required to identify the impacts on higher trophic levels, meta-community dynamics and the potential for legacy effects generated by successive low flows and heatwaves (Raquel, et al. 2021). Besides, other GHG are also important contributors to global warming. Arce demonstrated, in temperate IRES, while N2O emissions can be high at early drying, after long dry periods they tend to drop. Even under arid conditions, rewetting could foster N2O emissions (Arce, et al. 2018). How biofilms process carbon and nitrogen during the dry phase substantially influences ecosystem functioning and GHG export upon flow recovery, which leads us to understand better the role of intermittent rivers in GHG budgets at larger spatial scales. Moreover,

Given the increasing proportion of IRES affected by global climate change and human activity, they are becoming significant sources of CO2 and other GHG fluxes to the atmosphere. The duration and severity of the dry period are known factors influencing how C and N are processed (Schreckinger, et al. 2021). Although previous studies indicated that GHG emissions from dry riverbeds exposed to the atmosphere may be substantial, this process has not been rigorously quantified (Kosten, et al. 2018). Peak emissions at the onset of dehydration and the later rewetting should be quantified to obtain reliable emission estimates (Kosten, et al. 2018). Therefore, it is necessary to recognize the influence of increased dehydration duration on GHG emissions in IRES and it is necessary to integrate them into larger spatial and temporal scales.

4.3 Ecosystem multifunctionality of biofilms

4.3.1 Why estimate the ecosystem multifunctionality of biofilms

As an indicator of aquatic ecosystems' structural and functional coupling, biofilm plays an important role in the biogeochemical cycle, and the functions are not independent. Ecosystems can simultaneously provide multiple functions and services, termed multifunctionality or ecosystem multifunctionality (EMF) (Manning, et al. 2018). Ecosystems are often valued for the capacity to maintain multiple processes, yet most studies examined single processes in isolation (Naiara, et al. 2019). Evidence is mounting that external interference will trigger responses in different biofilm processes. For example, (Neif, et al. 2017) found that short-term stress events may have cascading effects on many important ecosystem processes. It is becoming increasingly clear that different species often influence different functions and that studying individual functions or processes, while isolating them may underestimate the level of biodiversity required to maintain multifunctionality.

IRES ecosystem functionality can be roughly divided into four main functions, such as material cycling, metabolism, ecological adjusting and providing habitats (Figure 5). The ecosystem provides habitats, nutrients, and energy for biofilms and other aquatic life, forming a unity of structure and functionality. Thus, lacking of research anyone is insufficient to fully reflect the structure and multifunctionality of biofilms. Even though those past studies provide enlightening insights into the effects of dehydration in IRES, most have focused on a single function at a time, rather than on the multitude of direct and indirect interactions between the networks of microbial taxa that co-exist in IRES. (de Vries, et al. 2018) argued that in the soil ecosystems, interactions and multifunctionality should be considered in the microbial communities, indicating a similar significance in IRES. Wagg also suggested that different microbes support different functions pointing to the significance of functional diversity within microbial communities (Wagg, et al. 2019). When multiple functions are considered in combination, the effect of diversity is different and even stronger (Byrnes, et al. 2014). If this combination between communities is ignored, our understanding of ecosystem function may be limited or even biased by current single-function approaches, because the functions observed in an ecosystem are usually interrelated. These correlations may be driven by common biological or abiotic drivers, interactions between functions, or trade-offs in the functioning of an individual organism due to physiological characteristics.

Recently, ecosystem multifunctionality has gained traction, but it has yet to be rigorously quantified, which is significant in understanding the mechanisms driving recovery from external interferences like dehydration. Especially given the sharp decline in global biodiversity, the correlation between biodiversity and ecosystem multifunctionality (BEMF) needs more profound research, which can help link with the effects on ecosystem stability (Pennekamp, et al. 2018). Therefore, when studying IRES, it is important to embrace the ecosystem's complexity and fully consider biofilm's multiple functions.

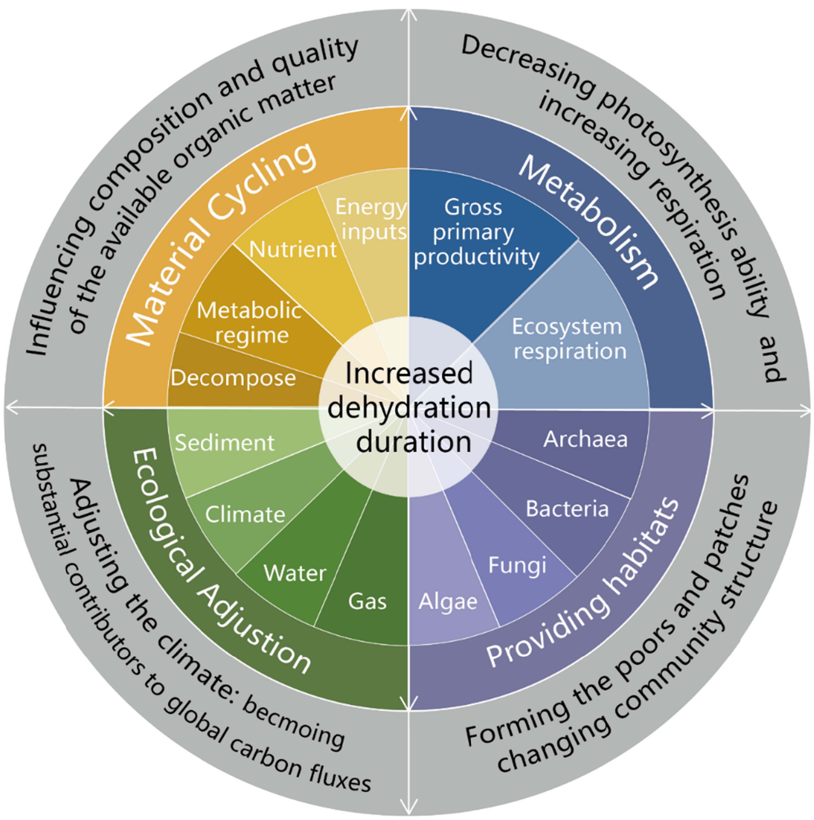

Figure 5. Multifunctionality of IRES ecosystem and the influence of increased dehydration duration. This diagram displays four main functions of IRES ecosystem, such as material cycling, metabolism, ecological adjusting and providing habitats. The ecosystem provides habitats, nutrients, and energies for biofilms and other aquatic life. These arrows imply that increased dehydration duration influences these lives by affecting the multifunctionality of the whole ecosystem.

4.3.2 How to estimate the ecosystem multifunctionality of biofilms

The relationship between biodiversity and ecosystem multifunctionality (BEMF) is a hot issue in current ecological studies. Ecosystems can provide multiple functions and services at the same time. It provides a more comprehensive evaluation of biological systems. In brief, a multifunctional approach allows for the weighting of individual functions and services from distinct categories (e.g. provisioning, regulating, supporting, and economic) and thus embraces the complexity of ecosystem trade-offs by decoupling "value" from individual response metrics (Custer and Dini-Andreote 2022). The measurement of ecosystem multifunctionality (EMF) is a crucial aspect of BEMF research, however, the metrics of EMF have been inconsistent among previous studies. Up to now, there have been seven approaches to qualifying EMF used by researchers. They are single function (Emmett Duffy, et al. 2003), turnover (Lefcheck, et al. 2015), averaging (Maestre, et al. 2012), single threshold method (Zavaleta, et al. 2010), multi-threshold method (Byrnes, et al. 2014), orthologue (Miki, et al. 2014), and multivariate model (Dooley, et al. 2015). Each one has its advantages, disadvantages and corresponding applicable conditions. Therefore, the selection of appropriate measurement methods is crucial to convincing research on ecosystem multifunctionality. Using multiple methods at the same time to learn from each other is a favored mode by researchers nowadays.

Despite the blooming and methodological approaches, our understanding of the IRES ecosystem is limited primarily due to their coupled aquatic–terrestrial characteristics (Rosetta, et al. 2021). Using innovative genomic tools such as high-throughput sequencing, qPCR, and meta-transcriptomic sequencing, is becoming a trend to understand the species, functions and adaptions in IRES. They are being used in combination will be a trend to polish and embellish the analysis of biofilm multifunctionality in IRES. While studies increasingly account for variations in abiotic conditions and functional composition when analyzing BEF relationships, these efforts are often quite moderate. It is largely unknown to what extent more integrative studies, accounting for all relevant abiotic and compositional factors and feedback from ecosystem functions to biodiversity, will alter our understanding of BEF relationships (Plas 2019).

Table 3. The measuring approaches of ecosystem multifunctionality. This table displays seven approaches to qualifying EMF and their advantages, disadvantages and corresponding applicable conditions.

No	Approaches	Calculation process	Advantages	Disadvantages	Reference
1	Single function	general linear model	It can be used as an auxiliary method.	The results are susceptible to individual species.	Emmett Duffy, et al. 2003
2	Turnover	Quantify the contribution of each species to EMF; Analyze EMF	It can reveal whether different species drive different ecosystem functions and can examine the importance of each species to different functions.	The procedure of data analysis is complicated. The weight of different functions is not considered.	Lefcheck, et al. 2015
3	Averaging	Translate, standardize and average	It is simple and intuitive and the results are easy to interpret.	It cannot distinguish differences in the importance of a species to different functions and the weight between functions is not easy to measure.	Maestre, et al. 2012
4	Single threshold method	calculate the number of functions in each ecosystem that reach a certain threshold and obtain an index to indicate the level of the overall function	More flexible and wide range of application	The results of a single threshold are often unconvincing and the dependencies and weights between functions are not considered.	Zavaleta, et al. 2010
5	Multi-threshold method	Calculate the number of functions that reach the threshold	It provides more information and is more flexible and comprehensive than Single threshold method	It is cumbersome and does not consider the correlation and weight between functions	Byrnes, et al. 2014
6	Orthologue	Gain orthologue cluster table from microbial genome database and convert it to a binary matrix	It can measure the functional gene diversity of a species, reflect the evolutionary diversity and study the BEMF relationship on a large scale	The prediction ability of multifunctionality is low and the application scope is small, so it can only be used for the analysis of multifunctionality of microbial community.	Miki, et al. 2014
7	Multivariate model	Transform the functional values to the same scale, fit, find the optimal model, and test	It can obtain relatively comprehensive information from multiple perspectives and quantify ecosystem versatility while analyzing individual functions	It is only suitable for studies with a small number of functions (e.g., 3 functions) and does not consider the weight of different functions.	Dooley, et al. 2015

5. Knowledge gap and outlook

This review summarized the effects of increased dehydration duration on the structure and functions of benthic biofilms in IRES. For the structure, the interactions network gives biofilms stronger vitality and makes them more complicated. Thus, employing co-occurrence network analysis on biofilm and exploring the differentiation of network patterns under dry-wet stress is vital to understand the whole ecosystem better. For the functions, despite the fundamental, the special ones, such as the emission of GHG deserves attention and can be a significant provider of the world river ecosystem. It is important to embrace the ecosystem's complexity and fully consider its multiple functions. Moreover, tools and approaches should be renovated to serve the purpose of more accurate and full-scale research in IRES ecosystem.

This review focused on the responses of biofilms to increased dehydration duration and highlighted the significance of ecosystem multifunctionality. Biofilms in IRES are of great significance to maintaining primary production, material circulation and energy flow. Their multifunctions in the alternation of dry-wet can be severely impacted by hydrological stress, with potential effects for ecosystem multifunctioning, including the contribution of GHG emissions. Now that the global extent of IRES is increasing and more unneglectable, our understanding of underlying adaptations that enable biofilms to persist in these highly dynamic changes is imperative to be expanded and deepened.

1. Each component of biofilm communities is indispensable because of the specific functions they perform in the ecosystem. Thus, more research is needed to reflect the structure and multifunctionality of biofilms fully. Communities' cooperation and interaction network are essential to forming biofilm structure and thus can't be ignored. When employing the co-occurrence network analysis, more and further diversity indexes besides α-diversity or β-diversity to understand the complex structure of microbial communities in future research.
2. It is ecosystem multifunctionality rather than a single function that needs to be fully considered when studying these biofilms and their functional roles under hydrological stress and other interference. That is because multifunctionality plays an essential role in the ecosystem, whose nature is proven to be multidimensional and comprehensive. To estimate multifunctionality more precisely, innovative genomic tools such as high-throughput sequencing, qPCR, and meta-transcriptomic sequencing are gaining traction. They are being used in combination will be a trend to polish and embellish the analysis of biofilm multifunctionality in IRES.
3. After dehydration, rewetting may bring a CO2 pulse, and flow recovery may make a new state rather than the original one. Whether the GHG flux recovers or not is to be confirmed by future research to understand better the role of intermittent rivers in GHG budgets at larger spatial scales.
4. The effects of prolonged dehydration may be underestimated because most research involved temporal snapshots at the local scale. More research is needed at

larger spatial and longer temporal scales to evaluate the effects from a more macro perspective. Furthermore, compared to the macro-external changes, a recent study indicated that intrinsic properties inside the IRES can also significantly influence the recovery of biofilms, even more than moisture content and history of intermittency.

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Author contributions

Bingling Yao, Chaoran Li and Lingzhan Miao designed the study and wrote the original draft. Tanveer M. Adyel, Ran Li, Jingjie Feng, Jun Wu and Jun Hou edited and revised the paper. All authors reviewed and commented on the paper.

Notes

The authors declare no competing financial interest.

Figure 1. The number of publications from Web of Science (from 1992 to 2023, updated to April, 2023). The data collection was conducted based on key words of the research of biofilms in IRES as well as dehydration.

Figure 2. Recurrent desiccation-rewetting cycles in IRES. Four phases exist in the cycles of IRES: aquatic phase, flow recession, flow recovery, and rewetting. Increased dehydration duration can impact both community structure and the multifunctional biofilm living in IRES, thus substantially affecting greenhouse gas fluxes.

Figure 3. Dynamic changes of metabolic functions of biofilms, including AI, ecosystem metabolism and carbon metabolism in IRES drying and rewetting period (Miao, et al. 2023). Different indexes showed distinct responses to short-term and long-term dehydration.

Figure 4. Dynamic changes of CO_2 fluxes in IRES drying and rewetting period. This diagram shows predictive CO_2 fluxes by IRES during different aquatic periods. When dehydration comes, metabolic functions begin to decline as well as GHG fluxes. During dryness, a peak may appear. After that, flow recovery may bring a CO_2 pulse which shows much higher flux and flow recovery may make a new state rather than the original one.

Figure 5. Multifunctionality of IRES ecosystem and the influence of increased dehydration duration. This diagram displays four main functions of IRES ecosystem, such as material cycling, metabolism, ecological adjusting and providing habitats. The ecosystem provides habitats, nutrients, and energies for biofilms and other aquatic life. These arrows imply that increased dehydration duration influences these lives by affecting the multifunctionality of

the whole ecosystem.

Table 1. Research progress of the microbial composition and community structure. The journals used in this survey have been published in recent five years in which researchers studied biofilms in IRES worldwide. They pointed out how increased dehydration duration influences the microbial composition and community structure.

Table 2. Research progress of the dynamic changes of biofilm function in IRES. This table displays responses of functional indicators to dehydration, implying dynamic changes in biofilm function in IRES worldwide.

Table 3. The measuring approaches of ecosystem multifunctionality. This table displays seven approaches to qualifying EMF and their advantages, disadvantages and corresponding applicable conditions.

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