Fungi and viruses as important players in microbial mats

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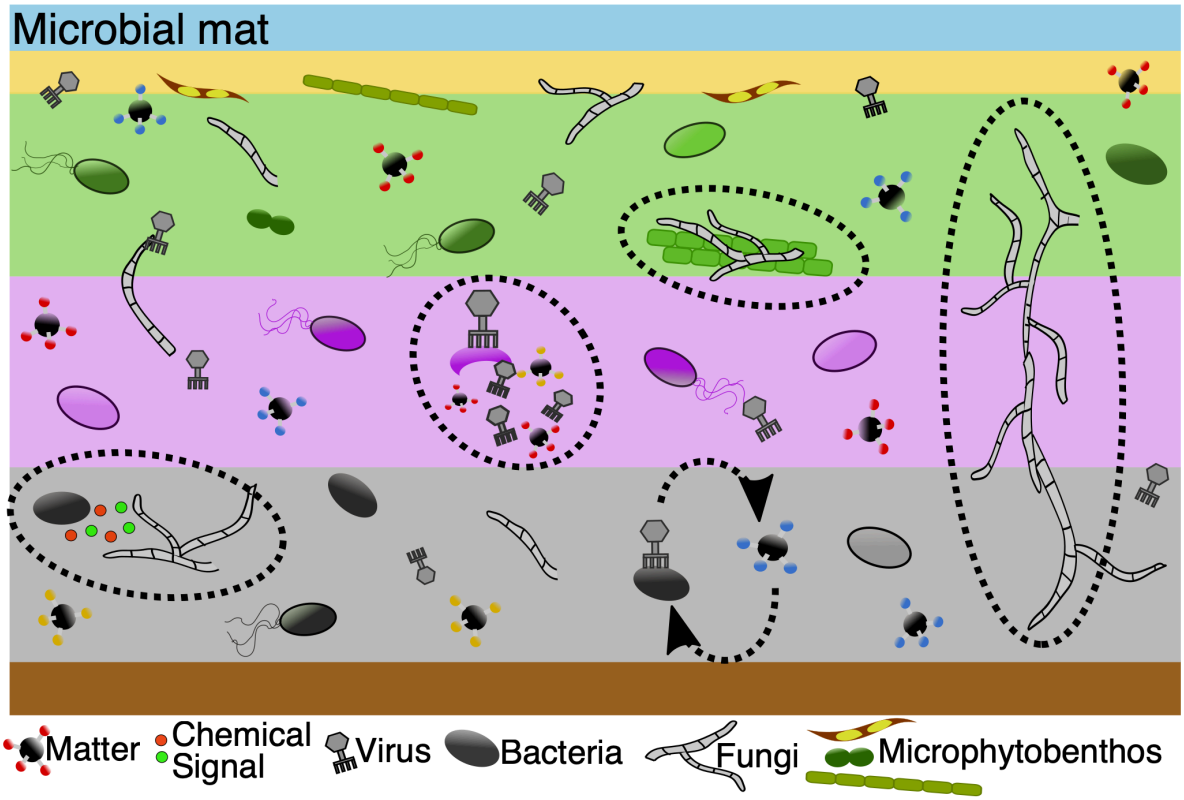

Abstract

Microbial mats are compacted, surface-associated microbial ecosystems reminiscent of the first living communities on early Earth. While often considered predominantly prokaryotic, recent findings show that both fungi and viruses are ubiquitous in microbial mats, albeit their functional roles remain unknown. Fungal research has mostly focused on terrestrial and freshwater ecosystems where fungi are known as important recyclers of organic matter, whereas viruses are exceptionally abundant and important in aquatic ecosystems. Here, viruses have shown to affect organic matter cycling and the diversity of microbial communities by facilitating horizontal gene transfer and cell lysis. We hypothesise fungi and viruses to have similar roles in microbial mats. Based on the analysis of previous research in terrestrial and aquatic ecosystems, we outline novel hypotheses proposing strong impacts of fungi and viruses on element cycling, food web structure and function in microbial mats, and outline experimental approaches for studies needed to understand these interactions.

Key words: microbial mats, fungi, virus, microbial food web

Glossary

Cable bacteria – Bacteria that conduct electrons over centimetre-long distances linking oxygen reduction at the sediment surface to sulphide oxidation in the deeper layers, thereby producing an electric current (Nielsen *et al.*, 2010, Schauer *et al.*, 2010).

Fungal highway – Refers to the continuous water films surrounding fungal hyphae enabling the movement of bacteria and reducing the average distance between bacteria and substrate (Kohlmeier *et al.*, 2005).

Kill the winner hypothesis – Refers to the co-existence of slow and fast-growing microalgae/bacteria competing for the same substrate as a result of viral infection preventing the most active populations from becoming dominant (Thingstad, 2000).

Lysogenic cycle – Occurs when the viral genomic material is incorporated into the host and is transmitted with every cell division (Weinbauer, 2004).

Lytic cycle – Occurs when the virus overtakes the host machinery, producing progeny, which is released into the environment (Weinbauer, 2004).

Microfluidics – Refers to the controlled manipulation of fluids at small scales allowing an accurate control of e.g. fluid flow, chemical gradients, and surface chemistry (Son *et al.*, 2015). In microbial ecology it has been used extensively in the study of microbial motility and chemotaxis.

Nanoscale secondary ion mass spectrometry (NanoSIMS) – Refers to a technique that separates ions of different mass to charge ratio which are then analysed in a mass spectrometer. This technique can detect one or more elements or isotopes simultaneously at a high spatial resolution (down to submicron-scale), and with high sensitivity allowing the imaging of metabolic activity within single cells (Pett-Ridge & Weber, 2012).

Nanoscale stable isotope probing (NanoSIP) – This technique goes a step further than NanoSIMS by mapping function and microbial identity, thus contextualising microbial roles in the community. It combines NanoSIMS-based stable isotope tracing with phylogenetic techniques (Pett-Ridge & Weber, 2012).

Nanoscope – Refers to a 50 nm resolution optical virtual imaging with a white-light nanoscope (Wang *et al.*, 2011).

Quorum Sensing – A type of chemical signalling used to coordinate activities, usually between cells.

Time-lapse fluorescence microscopy – This is a probabilistic approach that tracks viruses in a sequence of fluorescence microscopy images (Godinez *et al.*, 2007).

Viral shunt – Refers to when viral lysis of cells in aquatic ecosystems diverts the flow of matter away from higher trophic levels, and into the dissolved and detrital organic matter pools (Suttle, 2005).

Viral shuttle – Refers to when viral lysis enables the development of aggregates (e.g. marine snow) removing matter from the photic zone but increasing the carbon flux in the deep sea (Weinbauer, 2004).

Introduction

Microbial mats encompass microbes involved in all metabolic pathways existent on Earth and have remained surprisingly stable for over 3 billion years (Krumbein et al. 2003). Microbial mats represent the first ecosystems on Earth, and as such, they are important systems for understanding the emergence of life on and beyond the Earth (Fenchel, King and Blackburn 2012). They are also suggested to have modified the composition of the Earth's atmosphere, most notably the oxygenation of the atmosphere, greatly changing the geochemical and biological evolution (Hoehler, Bebout and Marais 2001). Microbial mats are therefore natural ecosystems where the development of microbial evolutionary processes, diversity and adaptation to environmental conditions can be observed (Ward et al. 1998; Villanueva et al. 2007; Inskeep et al. 2013).

Most microbes form natural aggregates that attach to surfaces described as biofilms (Flemming and Wuertz 2019). When these structures grow, and become complex systems, they are defined as microbial mats (Krumbein et al. 2003). The complexity of microbial mats derives from a dynamic relationship between microbial communities and physicochemical gradients. Microbial mats are typically found under extreme conditions (e.g. hot springs, arid or hypersaline environments) where grazing and bioturbation are low (or absent) (Kühl, Fenchel and Kazmierczak 2003; Fenchel, King and Blackburn 2012). Their stratified structure is often visible by eye, consisting of many ecological niches and microenvironments (Figs 1 and 2A). Typically, there is an upper light-exposed oxygenic layer composed of microalgae and cyanobacteria, but as light attenuates strongly in the densely pigmented layers, oxygen depletes rapidly due to microbial activity in the top few millimetres. In this anoxic zone, heterotrophic prokaryotes are forced to use a gradient of electron acceptors (manganese—Mn_4^+, nitrate—NO_3^-, iron— Fe_3^+, sulphate—SO_4^{2-} and carbon dioxide—CO_2) for anaerobic metabolism (Canfield, Thamdrup and Kristensen 2005), generating reduced reaction products such as sulphide. Sulphide in turn drives anoxygenic photosynthesis and chemolithotrophic sulphide oxidation facilitating sulphur cycling in the mat (Van Gemerden 1993). With depth, the cascade of microbial metabolic pathways typically ends with the anoxic black and grey layers, resulting fromthe activity of sulphate-reducing prokaryotes (Fig. 1). However, microbial mats can be quite diverse in their structure and therefore not all mats will show all the above-mentioned layers. For example, some microbial mats can be completely anoxic (Kobayashi et al. 2012).

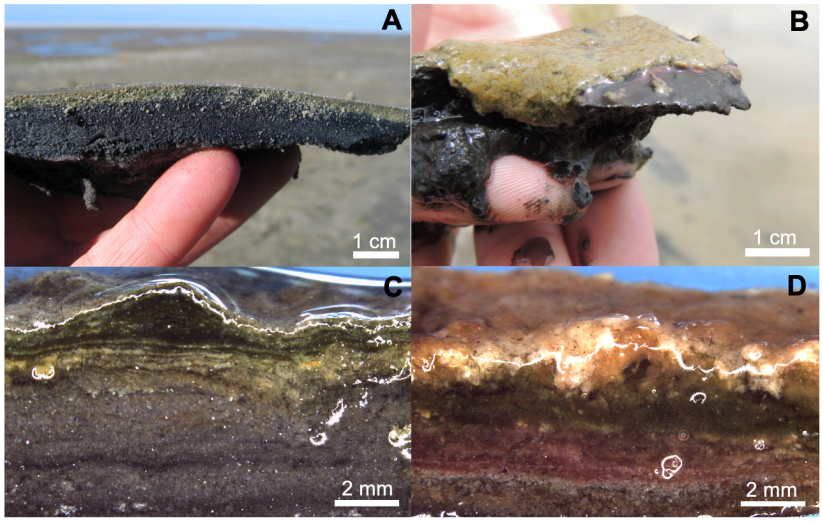

Figure 1. Examples of microbial mats. The photos show the fine layered structure with the top green oxygenic photosynthetic layer mainly composed of cyanobacteria and diatoms, followed by the pink anoxygenic photosynthetic layer (in B and D) and the grey/black sulphate-reducer layers. (A) Coastal microbial mat from the intertidal zone of the Wadden Sea island, Schiermonnikoog (The Netherlands); hypersaline microbial mats from (B) Laguna de Salinas (Arequipa, Peru) and (C, D) Salinas de Santiago (Aveiro, Portugal) with a salinity of 113 and 150, respectively. Note the salty yellow microbial layer in (D).

Sources of matter to microbial mats can come from sinking particles, but mostly originate from photosynthetic productivity within the mat ecosystem. There is a tight coupling between autotrophic and heterotrophic processes in microbial mats, and so most production is rapidly turned over and the net accretion rate is in the millimetres per year range. Bacteria (autotrophic and heterotrophic) are the main microbial groups comprising microbial mats, followed by archaea. When present, microalgae, e.g. diatoms, only comprise a minor component of the biomass within the upper mat layers (e.g. Bolhuis, Cretoiu and Stal 2014). However, fossil records (e.g. Krumbein et al. 2003) and recent studies (e.g. Cantrell and Duval-P ´erez 2013; Carreira et al. 2015a) have shown that fungi and viruses are also present in microbial mats.

Microscopic fungi (2–500 µm; hereafter fungi) are heterotrophic organisms made of hyphae that branch as they grow, forming a mycelium. Fungal exoenzymes break down organic matter outside the cells, which is then absorbed through the mycelium. Fungi can reproduce asexually or sexually, which is triggered by changes in environmental conditions. The capacity of fungi to degrade organic matter in terrestrial environments, where fungi are known to be abundant and diverse (Sigee 2005), makes them key players in elemental cycling and shaping the microbial diversity (e.g. Sigee 2005; Gadd 2007). Another ecological role of fungi has been shown in freshwater systems, where phytoplankton (e.g. colonial diatoms), unable to be digested by zooplankton, are first infected and consumed by parasitic fungal

zoospores (chytrids), which then are grazed by zooplankton (Kagami, Miki and Takimoto 2014). This 'mycoloop' avoids a loss of phytoplankton matter that would otherwise sink.

Viruses are small (20–1000 nm) obligate parasites composed of Deoxyribonucleic acid (DNA) or Ribonucleic acid (RNA) enveloped in a protein capsid with varying shapes. Viruses cannot move, respire or grow; hence, they must infect a host and manipulate its machinery and metabolism to replicate. The new viruses are then released by bursting the host cell (lytic cycle). However, the viral genome can also be incorporated into the host cell genome and replicate with each cellular division (lysogenic cycle). Eventually, lysogenic viruses can switch to a lytic cycle (Weinbauer 2004). Viruses are thus key agents in the transfer/exchange of genetic material across microbes, and aquatic viruses have been shown to strongly impact biogeochemical cycles and the diversity of microbial communities (e.g. Brussaard et al. 2008).

Considering that fungi and viruses ubiquitously influence ecosystem's flow of matter, diversity and productivity (Sigee 2005; Brussaard et al. 2008), we hypothesise a similar role in microbial mats, but this topic remains underexplored. Here, we present an overview of the current knowledge and discuss the potential role of fungi and virus in microbial mats.

Evidence of the potential role of fungi and viruses in microbial mats

Fungi can degrade organic matter (Cantrell and Duval-Pérez 2013), including cyanobacteria in microbial mats (Carreira et al. 2015b; Velázquez et al. 2016). High viral abundances (10^{10} g^{-1}; Carreira et al. 2015a) and diversity (Pacton et al. 2014) in microbial mats also suggest that viruses play an important ecological role. However, this role might have been overlooked, as suggested by two studies showing that viruses in microbial mats can be transformed into nanosized structures, probably misidentified as nanobacteria, in the process of calcification and lithification of microbial mats (Pacton et al. 2014; Wit et al. 2015). Both fungi and viruses have been detected in several types of mats (Tables 1 and 2), but there is the possibility that fungi might not be present in all microbial mats. Nonetheless, both fungi and viruses could (i) change the microbial community composition and diversity due to fungal interactions with prokaryotes (production of chemical signals, competition for resources), predation on microbial photoautotrophs (fungi) or by lysing (virus) specific communities/species/strains; (ii) change the productivity and flow of organic matter and nutrients, making it accessible to other microbes, due to substrate decomposition, microbial predation and competition with prokaryotes for resources (fungi), and cell lysis (virus); (iii) help maintain microbial mat stratification by providing (recycled) matter and retaining it within each vertical layer (virus); and (iv) facilitate the cycling and exchange of substrates between layers in the microbial mat due to mycelia growth (fungi). Understanding these interactions could reveal microbial mats as far more dynamic in terms of productivity, composition and

distribution patterns than previously thought (Fig. 2). In the next subsections, we will describe and discuss these points in more detail.

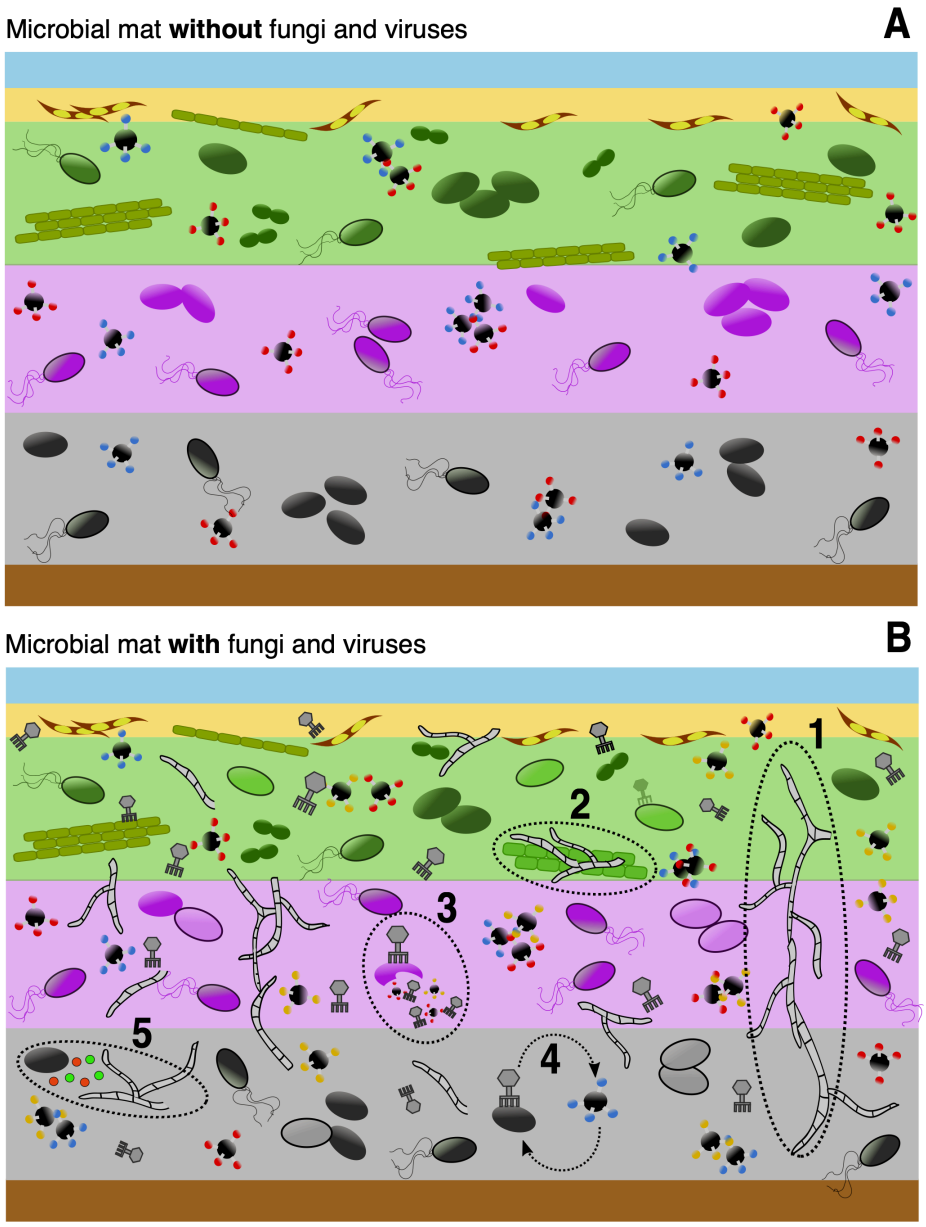

Figure 2. Fungi and viruses in microbial mats. Schematic representation of a typical stratified hypersaline microbial mat with microalgae and cyanobacteria (green layer), purple sulphur prokaryotes (pink layer), and sulphate-reducing prokaryotes (grey/black layer), without (A) and with (B) the inclusion of fungi and viruses. Numbers 1 to 5 display examples of suggested fungal and viral interactions in microbial mats: 1) fungi linking layers; 2) fungal predation on filamentous cyanobacteria; 3) viral infection of a prokaryote and subsequent release of viruses and organic matter; 4) viral-mediated recycling of organic matter and retention within each layer; and 5) chemical signalling between prokaryote and a fungus. The examples of interactions shown are not confined to the layers where they appear. An increased diversity in microbial and chemical compounds is indicated in B compared to A. The drawings are an artistic illustration and thus not to scale.

Do fungal predation/remineralization and viral lysis impact microbial mats diversity and function?

We suggest that fungi could have three main roles in microbial mats: (i) remineralise refractory organic matter and nutrients, facilitating further breakdown by microbes, (ii) trap organic matter and nutrients as a result of predation/attack of microbes, and (iii) impact microbial succession and diversity. Several mechanisms could contribute to these roles as described below. Fungi were observed in microbial mats in the late 1980s (e.g. Giani et al. 1989). However, it took nearly 30 years before they were clearly identified (Table 1) and it was shown that the presence of both fungi and bacteria in a hypersaline microbial mat allowed a faster degradation of organic matter, as compared with either group alone (Cantrell and Duval-Pérez 2013). Additionally, a recent study by Fabian et al. (2017) in a freshwater sediment showed that when occurring together with fungi, bacteria preferably degraded labile organic matter, while the fungi degraded the refractory part. Importantly, the recycled carbon from fungal activity fuelled mainly bacteria that either respired the carbon or incorporated it into biomass. Fungi could thus be an active component of microbial mats, degrading organic matter (possibly refractory parts) together with prokaryotes, while fuelling the microbial community. In freshwater, it is also well ocumented that fungal parasites control microalgae and cyanobacteria populations (Rasconi, Jobard and Sime-Ngando 2011 and references therein). In microbial mats, it has also been shown that fungi predate on cyanobacteria, changing the spatial structure of the microbial photoautotrophs (Carreira et al. 2015b), but the mechanism of predation and its impacts on biogeochemical cycling are currently unknown.

The interaction between bacteria and fungi can be modulated by chemical signalling that stimulates/inhibits microbial activity and growth (Deveau et al. 2018). Most research on chemical signalling in aquatic environments has focused on bacteria–bacteria interactions, while bacteria–fungi interactions are mostly investigated in soil science. Impacts of chemical signalling between fungi and bacteria range from plain presence to cooperation and obstruction (Deveau et al. 2018 and references therein). As microbial mats are hotspots for microbial chemical interactions (Decho, Norman and Visscher 2010), bacteria–fungi interactions might be an important, hitherto ignored part of element cycling in these compacted communities. Recently, temperate viruses have also been shown to use chemical signals (quorum sensing) to communicate on whether to remain lysogenic or start a lytic cycle under controlled laboratory conditions (Erez et al. 2017). This highlights the potential importance of searching for these interactions between microbes and viruses in microbial mats to understand their role and impact.

Microbes, in particular bacteria, are the most common viral hosts in seawater, which upon lysis release organic matter and nutrients to the environment. This impacts marine biogeochemistry by recycling organic matter and nutrients via the ‘viral shunt’ (Brussaard et

al. 2008; Lønborg, Middelboe and Brussaard 2013), or by increasing sequestration of organic matter to the deep sea as the lysates aggregate via the 'viral shuttle' (Lønborg, Middelboe and Brussaard 2013; Sullivan, Weitz and Wilhelm 2017). Viral abundances and infection rates can also be high in sediments (e.g. Danovaro et al. 2008; Carreira et al. 2013), with recent work showing infectivity down to several metres below the sea floor (Cai et al. 2019). Viruses also impact the diversity of microbial communities ('kill the winner'; Thingstad 2000) by enabling the co-existence of slow- and fast-growing hosts competing for the same limiting substrate. This has been suggested in several studies of model systems (Thingstad et al. 2014), laboratory experiments (Winter et al. 2004) and mesocosms (Sandaa et al. 2009). In aquatic systems, viral infections are tightly linked with microbes (Wigington et al. 2016), most commonly bacteria, but recently it has been suggested that viruses also infect fungi (Hassett et al. 2019). High viral abundances have also been observed in microbial mats (Carreira et al. 2015a) (Table 2), suggesting high infection rates and a role of viruses in the biogeochemistry of microbial mats. Currently, it is not clear whether viruses preferentially infect a group versus another in microbial mats, as this research area is still in its infancy.

Although some knowledge has been gained in marine and freshwater ecosystems regarding the importance of fungi and viruses, studies in microbial mats are still lacking. Therefore, to understand whether fungal predation/remineralisation and viral lysis impact microbial mat diversity and function, an essential first step is to study natural microbial mats from a fungi and virus perspective, for example, by determining their distribution, diversity, function and abundance in the mat and in relation to the other microbes. Metagenomics has been used mainly to determine the diversity and function of prokaryotes in microbial mats (e.g. Bolhuis, Cretoiu and Stal 2014; Ruvindy et al. 2016), but it is also possible to use this technique to identify fungi and viruses and their potential function in microbial mats (Cantrell and Baez-Félix 2010; Pacton et al. 2014). Such tools could be combined with microscopy, flow cytometry and imaging to determine fungal, viral and microbial abundances and their distribution (e.g. Carreira et al. 2015c,d).

A next step in revealing the role of fungi and viruses is to isolate key fungi and virus–host model systems from microbial mats to perform detailed studies under controlled conditions to understand the mechanisms of fungal predation and viral lysis, and the consequences of these processes for productivity and diversity of microbial mats. Determination of infection/predation and productivity rates would be essential, particularly at the scales significant to microbial ecology (µm–mm scale). Live imaging would be an interesting technique (Son, Brumley and Stocker 2015) to further our understanding of infection/predation rates coupled with physiology experiments. For example, a nanoscope or time-lapse fluorescence microscopy could be used to visualise live viral infections (Godinez et al. 2007; Wang et al. 2011). Other relevant techniques could encompass nanoscale

secondary ion mass spectrometry (NanoSIMS) to trace metabolic activity or nanoscale stable isotope probing (NanoSIP) that maps both function and microbial identity (Pett-Ridge and Weber 2012). These could be used to successfully link biogeochemical processes with specific microbial populations, and detect controls of incorporation and transfer of matter (e.g. carbon, nitrogen) between microbes and virus. Furthermore, these techniques could be used to label cells/viruses and incubate these with other groups, to reveal sharing, competition and exchange of matter between different groups (Mayali 2020).

Table 1. List of microbial mats locations and types where fungi were found with information on the taxon ('Present' indicates fungal presence but without taxonomic description), name, and reference. Information is listed by year of publication.

Location	Type of mat	Taxon	Name	Reference
Saltern, Kervalet, France	Hypersaline	Present		Giani et al. 1989
Movile Cave, Mangalia, Romania	Sulfurous cave springs	Class	Oomycetes	Sarbu et al. 1994
		Species	*Gliocladium* sp.	
		Species	*Penicillium* sp.	
		Species	*Plasmopora* sp.	
		Species	*Trichoderma* sp.	
Cabo Rojo Solar Salterns, Punta Los Morrillos, Puerto Rico	Hypersaline	Family	Sterile mycelium ''Dematiaceae''	Cantrell et. al. 2006
		Family	Sterile mycelium ''Moniliaceae''	
		Species	*Aspergillus carneus*	
		Species	*Aspergillus flavus*	
		Species	*Aspergillus heteromorphus*	
		Species	*Aspergillus nidulans*	
		Species	*Aspergillus niger*	
		Species	*Aspergillus* sp.	
		Species	Chaetomium globosum	
		Species	*Cladosporium cladosporioides*	
		Species	*Cladosporium sphaerospermum*	
		Species	*Nigrospora sphaerica*	
		Species	*Penicillium* sp.	
		Species	*Phialophora* sp.	
		Species	*Scopulariopsis* sp.	
		Species	*Sporothrix* sp.	

Guerrero Negro, Mexico	Hypersaline	Species	*Metschnikowia bicuspidata*	Feazel et al. 2008
Hamelin Pool, Shark Bay, Australia	Hypersaline stromatolites	Species	*Engyodontium album*	Allen et al. 2009
Larsemann Hills, Vestfold Hills, & McMurdo Dry Valleys, Antarctica	Cold	Genus	*Acremonium*	Brunati et al. 2009
		Genus	*Alternaria*	
		Genus	*Arthrinium*	
		Genus	*Beauveria*	
		Genus	*Botrytis*	
		Genus	*Curvularia*	
		Genus	*Embellisia*	
		Genus	*Geomyces*	
		Genus	*Penicillium*	
		Genus	*Phialophora*	
		Genus	*Phoma*	
		Genus	*Thelebolus*	
		Species	*Aspergillus clavatus*	
		Species	*Aspergillus niger*	
		Species	*Candida lipolytica*	
		Species	*Cladosporium herbarum*	
		Species	*Cladosporium*	
		Species	*Cryptococcus albidus*	
		Species	*Cryptococcus infirmo-miniatus*	
		Species	*Cryptococcus laurentii*	
		Species	*Debaryomyces hansenii var. hansenii*	
		Species	*Geomyces pannorum*	
		Species	*Leucosporidium antarcticum*	

		Species	*Leucosporidium scottii*	
		Species	*Mrakia frigida*	
		Species	*Penicillium chrysogenum*	
		Species	*Penicillium crustosum*	
		Species	*Penicillium rugulosum*	
		Species	*Rhodotorula aurantiaca*	
		Species	*Rhodotorula minuta*	
		Species	*Rhodotorula mucilaginosa*	
Clear Creek, Coloroado, USA	Iron-rich	Class	Dothideomycete (possibly *Phaeosphaeria* sp.)	Gerea et al. 2012
		Class	Glomeromycete (possibly *Entrophospora* sp.)	
Cabo Rojo Solar Salterns, Punta Los Morrillos, Puerto Rico	Hypersaline	Species	*Acremonium sp.*	Cantrell and Duval-Peérez 2013
Schiermonnikoog, The Netherlands	Intertidal	Species	*Emericellopsis* sp.	Carreira et al. 2015
Ward Hunt Lake & stream at Taconite Inlet, Canada	Cold	Present		Rios et al. 2015
Livingston Island, Antarctica	Cold	Division	Basidiomycota	Velázquez et al. 2016
Salar de Llmara, Atacama Desert, Chile	Hypersaline	Division	Ascomycota	Saghaï et al. 2017
		Division	Basidiomycota	
		Division	Chytridiomycota	
Lagunita, Cuatro Cienegas Basin, Mexico	Wetland	Order	Diaporthales	Anda et al. 2018
Salar de Llmara, Atacama Desert, Chile	Hypersaline	Genus	*Fusarium*	Santiago et al. 2018

Are fungal predation/remineralization and viral infections in microbial mats limited to specific niches with certain physicochemical conditions?

Fungi are unique among eukaryotes due to their capacity to survive in extreme conditions. For example, fungi can withstand a wide range of temperatures and can be found in the cold deserts in Antarctica as well as in thermal springs (Gunde-Cimerman et al. 2004; Bazarzhapov et al. 2006). Fungi are also able to tolerate high salinities, and show a high taxonomical diversity, e.g. in salterns growing at NaCl concentrations>17% (average seawater is normally around 3.5%; Gunde-Cimerman et al. 2004; Cantrell, Casillas-Martínez and Molina 2006). Fungal growth has been observed in both inundated and dry microbial mats (Cantrell and Duval-Pérez 2013; Anda et al. 2018). Cantrell, Casillas-Martínez and Molina (2006) showed also a marked decrease in fungal abundance below the oxic layer in a hypersaline microbial mat, while other studies (Zhou et al. 2002; Jebaraj and Raghukumar 2009) found fungi in anoxic environments, where they survived by respiring nitrate (denitrification). These studies show that fungi may withstand different extreme conditions and may be a ubiquitous component of microbial mats.

Viruses have also been shown to persist under extreme environmental conditions. However, their occurrence is vastly dependent on the availability and survival of their hosts. UV radiation (specifically UV-B) has been shown to negatively impact viral infectivity in seawater (Mojica and Brussaard 2014) and a similar inhibitory effect can be expected in microbial mats that are often exposed to strong sunlight in the upper mat layers. We speculate that diel and/or seasonal changes in irradiance could be important regulators of viral infectivity in microbial mats. External stimuli, such as changes in environmental conditions (e.g. salinity, light), might switch viral infection from lysogenic to a lytic mode, and vice versa (Bettarel et al. 2011; Mojica and Brussaard 2014). Therefore, it is important to search for both lytic and lysogenic infection modes in microbial mats. The presence of viruses in microbial mats indicates that their ability to infect hosts remains, even under extreme conditions.

To decipher whether fungal predation/remineralisation and viral infections are limited to specific niches in microbial mats, initial studies could expose viral–host models, fungi and natural microbial mats to the typical extreme physicochemical conditions found in microbial mats (e.g. fluctuations in desiccation, irradiance, temperature and salinity). For example, culture experiments could determine the range of conditions limiting the viability of fungi and virus–host models by performing physiological measurements such as abundance, live/dead cells and active cells using flow cytometer and/or microscopy (e.g. Kwolek-Mirek and Zadrag-Tecza 2014; Gasol and Morán 2015). Subsequently, natural microbial mats could be incubated under similar environmental conditions, and fine-scaling sampling and measurements could reveal whether fungi and viruses are restricted to specific niches (e.g. oxygen and irradiance). For this, the use of techniques, such as fluorescence and confocal

microscopy and molecular markers (e.g. Hickey et al. 2004; Solé, Diestra and Esteve 2009; Adriaenssens and Cowan 2014; Grossart and Rojas-Jimenez 2016), would allow to evaluate the impact of environmental conditions on the distribution and activity of fungi and viruses. Microsensor technology could be used to determine physicochemical gradients (e.g. Kühl, Lassen and Jørgensen 1994; Epping, Khalili and Thar 1999).

What are the mechanisms of movement and transmission of viruses in microbial mats?

In microbial mats, microbes (and viruses) are embedded in a cohesive matrix of exopolymeric substances restricting movement. Viruses need to encounter new suitable hosts and several potential mechanisms could promote viral dispersion or their exposure to hosts in microbial mats. For example, viruses could be moved via advective flow through interstitial waters (Sutherland et al. 2004). Another way could be through electrostatic interactions that could either retain or repulse viruses (Sutherland et al. 2004) to surfaces (sediment particles, host, etc.). Viruses could also infect motile or migrating cells (e.g. cells with flagella, filamentous cyanobacteria); thus, we speculate that motile cells are more prone to viral infection, as compared with immobile cells in microbial mats. Finally, host cells could be induced by the virus to produce a viral biofilm whereby the newly formed virions aggregate on top of the infected cell, facilitating a more effective transmission after the infected cell contacts new cells, as suggested for human T-cells (Thoulouze and Alcover 2011). To address these challenging issues, dynamic imaging would be a fundamental approach. For example, real-time imaging (nanoscope and/or time-lapse microscopy) together with microfluidics could be used to observe viral dispersion within artificially laboratory grown biofilms (Godinez et al. 2007; Wang et al. 2011; Son, Brumley and Stocker 2015). Other approaches such as confocal and electron microscopy together with molecular markers could also be useful to determine viral dispersion directly inside the microbial mat (Solé, Diestra and Esteve 2009; Adriaenssens and Cowan 2014; Grossart and Rojas-Jimenez 2016).

Table 2. List of microbial mats locations and types where viruses were found with information on the rank ('Present' indicates viral presence but without taxonomic description), taxon, genome composition (single strand DNA - ssDNA, double strand DNA - ddDNA, single strand RNA - ssRNA, double strand RNA - dsRNA), and reference. Information is listed by year of publication.

Location	Type of mat	Rank	Taxon	Genome Composition	Reference
Pozas Azules II pool & Rio Mesquites River, Cuatro Cienegas Basin, Mexico (freshwater); Highborne Cay, Bahamas (marine)	Microbialites	Family	*Geminiviridae*	ssDNA	Desnues et al. 2008
		Family	Unclass. *Microviridae*	ssDNA	
		Subfamily	Unclass. *Gokushovirinae*	ssDNA	
			Unclassified	ssDNA	
		Genus	*Chlamydiamicrovirus*	ssDNA	
		Genus	*Bdellomicrovirus*	ssDNA	
		Genus	*Circovirus*	ssDNA	
		Genus	*Inovirus*	ssDNA	
Ward Hunt Ice Shelf, Markham Ice Shelf, & along the northern coastline of Ellesmere Island, Canada	Cold		Eukaryotic virus		Varin et al. 2010
			Bacteriophage		
Comau Fjord, Chile	Cold	Family	*Coronaviridae*	ssRNA	Ugalde et al. 2013
		Family	*Reoviridae*	dsRNA	
		Family	*Poxviridae*	dsDNA	
		Family	*Herpesviridae*	dsDNA	
		Family	*Baculoviridae*	dsDNA	
		Family	*Siphoviridae*	dsDNA	
		Family	*Podoviridae*	dsDNA	
		Family	*Myoviridae*	dsDNA	
		Family	*Adenoviridae*	dsDNA	
Lagoa Vermelha, Brazil	Hypersaline	Present			Pacton et al. 2014

Schiermonnikoog, The Netherlands	Intertidal	Present			Carreira et al. 2015
"La Salada de Chiprana", Spain	Hypersaline	Present		DNA and RNA	Wit et al. 2015
near Gobabeb Training and Research Station (Hosabes) & near Swakopmund (Eisfeld Playas), Namib Desert	Hypersaline	Family	*Circoviridae*	ssDNA	Adriaenssens et al. 2014
		Family	*Geminiviridae*	ssDNA	
		Family	*Inoviridae*	ssDNA	
		Family	*Microviridae*	ssDNA	
		Subfamily	*Gokushovirinae*	ssDNA	
			Unclass.	ssDNA	
		Order	*Caudovirales*	dsDNA	
		Family	*Myoviridae*	dsDNA	
Livingston Island, Antarctica Peninsula	Cold	Order	*Picornavirales*	ssRNA	Velázquez et al. 2016
		Order	Unclass. *Picornavirales*	ssRNA	
		Family	*Caliciviridae*	ssRNA	
		Family	*Dicistroviridae*	ssRNA	
		Family	*Iflaviridae*	ssRNA	
		Family	*Leviviridae*	ssRNA	
		Family	*Marnaviridae*	ssRNA	
		Family	*Nodaviridae*	ssRNA	
		Family	*Potyviridae*	ssRNA	
		Family	*Secoviridae*	ssRNA	
		Family	*Tombusviridae*	ssRNA	
		Genus	*Bacillariornavirus*	ssRNA	
		Genus	*Labyrnavirus*	ssRNA	
		Genus	*Ourmiavirus*	ssRNA	
		Family	*Partitiviridae*	dsRNA	
		Family	*Totiviridae*	dsRNA	

		Family	*Circoviridae*	ssDNA	
		Family	*Geminiviridae*	ssDNA	
		Family	*Inoviridae*	ssDNA	
		Family	*Microviridae*	ssDNA	
		Family	*Nanoviridae*	ssDNA	
		Family	*Parvoviridae*	ssDNA	
			Unclass.	ssDNA	
			Satellites		
		Family	*Baculoviridae*	dsDNA	
		Family	*Herpesviridae*	dsDNA	
		Family	*Iridoviridae*	dsDNA	
		Family	*Mimiviridae*	dsDNA	
		Family	*Myoviridae*	dsDNA	
		Family	*Phycodnaviridae*	dsDNA	
		Family	*Podoviridae*	dsDNA	
		Family	*Poxviridae*	dsDNA	
		Family	*Siphoviridae*	dsDNA	
			Unclass.	dsDNA	
			Unclass.	DNA	
Middle Island Sinkhole, Lake Huron, USA	Low Oxygen	Present			Voorhies et al. 2016
Lagunita, Cuatro Cienegas Basin, Mexico	Wetland	Present			Anda et al. 2018
Mesaieed sabkha, Qatar	Hypersaline	Present			Perri et al. 2018
Hamelin Pool, Shark Bay, Australia	Hypersaline stromatolites	Family	*Nanoviridae*	ssDNA	White et al. 2018
		Family	*Parvoviridae*	ssDNA	
		Family	Unclass. *Microviridae*	ssDNA	
		Family	Unclass. *Circoviridae*	ssDNA	
		Family	Unclass. *Inoviridae*	ssDNA	

			Family	Unclass. *Geminiviridae*	ssDNA
			Subfamiliy	*Bullavirinae*	ssDNA
			Subfamiliy	Unclass. *Gokushovirinae*	ssDNA
			Genus	*Bdellomicrovirus*	ssDNA
			Genus	*Begomovirus*	ssDNA
			Genus	*Chlamydiamicrovirus*	ssDNA
			Genus	*Circovirus*	ssDNA
			Genus	*Eragrovirus*	ssDNA
			Genus	*Inovirus*	ssDNA
			Genus	*Mastrevirus*	ssDNA
			Genus	*Pletrovirus*	ssDNA
				Unclassified Satellites	ssDNA
"Cone Pool" Little Hot Creek, California, USA	Hot spring	Present			Jarett et al. 2020

Could fungal mycelia connect distinct layers in microbial mats and impact the biogeochemistry across redox zonations?

Mycelia are essential in degradation and absorption of matter in soils and can extend from a few microns to several metres long, forming intricate networks (Islam et al. 2017). In soils, this capacity is essential for fungi to cope with environmental disturbances, variability in nutrient distribution, 'resource transport' from favourable to unfavourable locations allowing fungal growth and exploration of new locations, and enabling bacterial movement ('fungal highways'; Kohlmeier et al. 2005; Boswell et al. 2007; Fricker et al. 2017; Deveau et al. 2018). In microbial mats, we suggest that fungi could (i) access substrates from different layers (e.g. oxic and anoxic); (ii) survive despite daily fluctuations in the mat's chemical gradients and position of the substrates; (iii) transfer and exchange matter along different layers, alike 'cable bacteria' (Malkin and Meysman 2015), but instead of transferring electrons, mycelia would be transferring matter (molecules); and (iv) enable bacterial movements (Kohlmeier et al. 2005). Culture studies of single fungal species and microbial communities with additions of various sources of matter would enable the understanding of the role of fungal mycelia. NanoSIMS and microscopy (confocal and fluorescence) could be applied to follow the distribution of molecules and cells. These studies should be coupled with fine-scale sampling and microsensor profiles of microbial mats to determine the dispersion and activity along the fungal mycelia and in the layers of the mat.

Conclusion

Contrary to pelagic or terrestrial ecosystems where the food web spreads from simple organic matter to higher vertebrates, in most microbial mats, the food web is entirely microbial. The fact that both fungi and viruses are detected in microbial mats indicates a potentially important role in the biogeochemistry. As a way forward in understanding the importance of these additional components, we have presented some hypotheses and examples of studies and techniques, which could provide first clues about their role, but also spark further discussion that would stimulate new ideas and advances.

The apparent lack of observations of fungi in microbial mats could be compared with a similar problem observed in marine ecosystems, where the role of fungi has been largely ignored, with a few recent exceptions (e.g. Jobard, Rasconi and Sime-Ngando 2010; Richards et al. 2012). This lack of focus on marine fungi is suggested to be the result of several factors such as the absence of knowledge and therefore appropriate targeting of fungi when studying marine samples (Richards et al. 2012). It has also been suggested that a lack of appropriate marine fungal genomic libraries is due to bias towards terrestrial or cultured fungi, potentially underestimating or missing marine fungal diversity (Richards et al. 2012; Grossart et al. 2016). Misidentification of fungi due to methodological issues or mistaking them for other organisms

could also hinder their proper identification (Grossart et al. 2016; Frenken et al. 2017). Improving our awareness of the role of fungi generally might lead to greater efforts for a correct identification, and subsequent inclusion of fungi as players in the dynamics of microbial mats.

The impact of global change on the dynamic interactions of the microbial and viral community in microbial mats is currently unknown. Although cyanobacterial hypersaline microbial mats have been suggested as potential short- and long-term indicators of climate change (Paerl et al. 2003), there are only a few studies that attempted to determine the impact of global change on microbial mats. Kleinteich et al. (2012) showed increased cyanobacterial diversity and toxins with warming in polar freshwater microbial mats after 6 months of elevated temperatures. However, other studies found no changes in microbial mats under varying global change conditions. For example, lithifying microbial mats exposed to high CO2 (1200 ppm) for 6 months did not show change in the microbial diversity (Ahrendt et al. 2014). Lowering the salinity levels on a hypersaline microbial mat over the course of a year also did not impact the microbial community composition (Green et al. 2008). These results together with the high diversity of microbes, interactions and functions found in microbial mats seem to indicate a resilience to disturbances (De Anda et al. 2018). Nonetheless, global change could limit/extend the habitat availability. For example, polar microbial mats could lose habitat due to warming (Vincent, Gibson and Jeffries 2001).

Overall, the development of new/improved methods to measure, e.g. in situ mortality and production caused by fungal predation/remineralisation and viral lysis, would allow a better understanding of the individual and combined effects of fungi and viruses in microbial mats. Here, it would be vital to look further into other research areas, such as medical and biofilm research. For example, time-lapse fluorescence microscopy that was here proposed as a tool to observe live viral infections is already used in biomedical research (Godinez et al. 2007). Strong interdisciplinary efforts are needed to join these complementary research fields, with the outcomes potentially offering major advances in our understanding of microbial mats.

On the whole, fungi and viruses could allow trapping and/or supply matter at specific depths, and be evolutionary drivers, promoting genetic diversity, by constantly changing the microbial mat's structure. However, experimental results are necessary to show the qualitative and quantitative importance of fungi and viruses in the complex but stable microbial mat's ecosystems reminiscent of the early Earth.

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