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► To cite this version:

Hugo Sentenac, Adeline Loyau, Joséphine Leflaive, Dirk S Schmeller. The significance of biofilms to human, animal, plant and ecosystem health. *Functional Ecology*, 2022, 36 (2), pp.294-313. 10.1111/1365-2435.13947 . hal-04210839

HAL Id: hal-04210839

<https://hal.science/hal-04210839v1>

Submitted on 19 Sep 2023

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1 The significance of biofilms to human, animal, 2 plant and ecosystem health

3 Hugo Sentenac^{a,*}, Adeline Loyau^{a,b}, Joséphine Leflaive^a, Dirk S. Schmeller^a

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5 ^aLaboratoire Ecologie Fonctionnelle et Environnement, UMR 5245, Université de Toulouse,
6 CNRS, INPT, UPS, Avenue de l'Agrobiopole, BP32607 Auzeville-Tolosane 31126 Castanet-
7 Tolosan Cedex, France

8 ^bDepartment of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland
9 Fisheries (IGB), Alte Fischerhütte 2, Stechlin, D-16775, Germany

10 *Correspondence: hugo.sentenac@toulouse-inp.fr, +33 (0)6 37 07 68 72

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12 This is a preprint version. Please consult the final peer-reviewed version of this manuscript,

13 published in Functional Ecology with DOI [10.1111/1365-2435.13947](https://doi.org/10.1111/1365-2435.13947)

14 Abstract

15 1. Biofilms are matrix-enclosed communities that represent the most dominant and
16 active mode of microbial life on Earth. Because biofilms are inherently more
17 productive than any equivalent planktonic community, they are of great relevance
18 to all environments they inhabit. However, their existence and importance are still
19 poorly known by the general public, conservation practitioners, and
20 environmental policymakers.

21 2. Most microorganisms of multicellular organisms (including humans, animals and
22 plants) occur in the form of true biofilms or biofilm-like structures that play vital
23 roles in their development, physiology and immunity. Conversely, some biofilms
24 can have a negative effect on host health.

25 3. Biofilms growing on non-biological surfaces are essential components of many
26 terrestrial and marine ecosystems: they form the basis of food webs and ensure
27 nutrient cycling and bioremediation in natural systems. However, environmental
28 biofilms can promote the persistence of human pathogens, produce harmful
29 toxins, foul and corrode surfaces in natural and man-made settings; all of which
30 can have significant health and economic implications.

- 31 4. There is a knowledge gap about the roles of biofilms in the epidemiology of
32 wildlife emerging infectious diseases, yet these pose a major threat to public
33 health, biodiversity and sustainability. The drivers of global environmental change
34 all affect biofilm structure and functions. The consequences for host and
35 ecosystem health are, however, poorly understood. While the concept of a healthy
36 microbiome (as opposed to dysbiosis) is emerging in medicine and conservation
37 biology, the concept of a healthy biofilm remains to be defined in environmental
38 sciences.
- 39 5. Here, we use an integrative approach to (i) review current knowledge on the roles
40 of biofilms growing on biological and non-biological interfaces for the health of
41 multicellular organisms and ecosystems, and (ii) provide future research
42 directions to address identified knowledge gaps. Giving the biofilm life form its
43 full importance will help understand the effects of global environmental change
44 on these communities and, in turn, on human, animal, plant and ecosystem health.

45 **Keywords**

46 Antibiotic resistance, Detoxification, Disease Pyramid, Environmental monitoring,
47 Holobiont, Freshwater ecosystems, One Health, Periphyton

48 **1. Introduction**

49 The overall importance of microorganisms for the health of multicellular hosts and
50 ecosystems is becoming increasingly evident (Bernardo-Cravo et al., 2020; Cavicchioli et al.,
51 2019; Clemente et al., 2012). The advent of new technologies such as high-throughput
52 sequencing made the study of the specific diversity of microorganisms (the "microbiota"),
53 their genomes and their surrounding environment, which together form the microbiome,
54 widely available (Marchesi and Ravel, 2015). Microorganisms are now considered the
55 bedrock of biodiversity, playing a pivotal role in global change biology (Cavicchioli et al.,
56 2019; Zhu and Penuelas, 2020). Yet, the fact that most of them live in complex communities
57 attached to an interface in a three-dimensional gel-like matrix, known as biofilms (Flemming
58 and Wuertz, 2019), is still poorly recognised in research, conservation practices, and
59 environmental policies. We believe this oversight hinders a good grasp of the relationships
60 between the structures and functions of microbial communities in various environments, thus
61 limiting a thorough understanding of the determinants of host and ecosystem health.

62 Compared to planktonic microorganisms, the biofilm is a very different life form: the
63 gel-like matrix allows for the retention of water and nutrients, and also provides its
64 inhabitants with protection against physical, chemical or biological aggressions and changing
65 environmental conditions (Flemming et al., 2016; Flemming and Wingender, 2010). This
66 temporal and physical stability likely explains why biofilms were found to be one of the
67 oldest, dating back 3.3 billion years ago (Westall et al., 2001), and most successful life forms,
68 prevailing in almost all habitats on Earth (Battin et al., 2016; Flemming and Wuertz, 2019).
69 Biofilm research is located at the nexus of many fields of life sciences, including
70 microbiology and functional ecology (Battin et al., 2003), biodiversity and evolution
71 (Besemer, 2015; Hansen et al., 2007), biogeochemistry (Battin et al., 2016), biotechnology
72 (Edwards and Kjellerup, 2013), human and veterinary medicines, and public health
73 (Clutterbuck et al., 2007; Costerton et al., 1999; Mah and O'Toole, 2001). Biofilms provide,
74 therefore, an ideal opportunity for transdisciplinary and integrative research, for instance in
75 health-related sectors (Queenan et al., 2017). In the last decades, several fully-integrated
76 health approaches have emerged (Supplementary Fig. S1), including the convergent One
77 Health and the Ecosystem Health (EcoHealth) initiatives (Zinsstag, 2012), within which
78 health is understood in its broadest sense, including physical, moral, and social well-being,
79 and not only as the mere absence of disease. Health has strong socio-ecological determinants
80 and is therefore intimately linked to ecosystem health, sustainability and global change
81 (Supplementary Fig. S1). A healthy ecosystem is defined here as a **productive** system,
82 **resilient** against external pressures, and capable of maintaining its **structure** and services
83 to humans (Rapport et al., 1998; Tett et al., 2013).

84 However, to date, there is no comprehensive review on the numerous health
85 implications of the biofilm life form for humans, animals, plants and ecosystems. Because
86 biofilm research is very disparate (Supplementary Fig. S2), we deem here that using a holistic
87 approach to health is appropriate for analysing the all-encompassing relevance of biofilms. In
88 doing so, we demonstrate their global significance, unite the highly contrasting fields of
89 biofilm research, and identify knowledge gaps and areas for further biofilm investigations that
90 will contribute to improving our understanding of host and ecosystem health. We also provide
91 a definition of a healthy biofilm, which we believe is essential to properly detect, monitor and
92 mitigate the effects of global change on these communities.

93 **2. Definition and properties of biofilms**

94 Biofilms are generally defined as matrix-enclosed populations or communities of
95 organisms adherent to an interface (usually solid–liquid, but also liquid–liquid, liquid–gas and
96 solid–gas) and/or to each other (i.e. non-attached aggregates of cells, often termed flocs;
97 Costerton et al., 1987; Hall-Stoodley et al., 2004). In the strictest sense (hereafter biofilm
98 *sensu stricto*), the matrix in which biofilm microorganisms live is self-produced and made out
99 of extracellular polymeric substances (EPS, Vert et al., 2012). These EPS contain
100 polysaccharides, nucleic acids, proteins, lipids, other carbohydrates, and any element
101 pertaining to the medium in which biofilms occur (Flemming and Wingender, 2010).
102 Flemming and Wuertz (2019) expanded the definition of biofilms (hereafter biofilm *sensu*
103 *lato*) to include the mucosa-associated microbiota of animals, whose epithelia may produce
104 mucus (e.g. on the lining of the digestive tract or on the skin). Contrary to biofilms *sensu*
105 *stricto*, polysaccharides in biofilms *sensu lato* are secreted by the host glands and not by
106 microorganisms. However, this broad definition of biofilms has the advantage of drawing a
107 clear line between, on the one hand, microorganisms living aggregated in a matrix, and on the
108 other hand, planktonic (‘non-sessile’) or attached individual microorganisms (Flemming and
109 Wuertz, 2019). The continuum between a single attached cell and the different types of
110 microbial aggregates forms an **ecological succession** (Fig. 1A).

111 Biofilm research has long lacked a theoretical framework, but now is solidly rooted in
112 ecological theory built upon work from Battin et al. (2007). Landscape and
113 **metacommunity** ecology are now central tenets of biofilm research, which help
114 understand community assembly and the processes generating biodiversity within biofilm and
115 how this relates to biofilm functioning, productivity and resilience (Battin et al., 2007;
116 Besemer, 2015; Besemer et al., 2012; Nemergut et al., 2013). Not only does ecological theory
117 help understand biofilm biology, but biofilm research in turn can also help inform ecological
118 theory (Feng et al., 2017; Flemming et al., 2016; Prosser et al., 2007). There are, as a matter
119 of fact, many similarities between biofilms, forests or corals: all form biogenic habitat,
120 providing a 3-D structure and a somewhat enclosed environment within which processes and
121 internal interactions are enhanced, and through which external interactions are filtered (e.g.
122 resources or invasive species; Flemming et al., 2016). Therefore, insights from macroecology
123 has considerably improved the way biofilms are understood, but in turn, biofilms provide a
124 model to understand **emergent properties** of biological systems and also the opportunity
125 to test hypotheses (which can sometimes be inaccessible from the study of other organisms;

126 e.g. the diversity-stability hypothesis), and the roles of different ecological
127 interactions in the structuring of communities (Battin et al., 2007; Flemming et al., 2016;
128 Ponge, 2005).

129 Doubtless, the most distinctive feature of a biofilm is the presence of a 3-D matrix that
130 allows a steady juxtaposition of microorganisms next to one another for prolonged periods of
131 time (Flemming et al., 2016). As a result, biofilms are more organised and productive than
132 any equivalent planktonic community (Hansen et al., 2007): the biomass (productivity) of the
133 Archaea and Bacteria cells in biofilms was conservatively estimated at 4 to 11% of the total
134 biomass on Earth (see supplementary materials; Flemming and Wingender, 2010; Lock et al.,
135 1984). Biofilms exhibit emergent properties such as enhanced horizontal gene transfer,
136 including that of antimicrobial resistant genes, but also active sorption (capture, facilitated by
137 streamers), transport (through pores, voids, and channels) and extracellular retention of water,
138 nutrients, and enzymes (Fig. 1B). Above all, the cooperation between cells (social
139 interactions), through chemical and electrical communication, may be the most surprising
140 property, with signs of division of labour and coordinated behaviour, thought to be typical of
141 multicellular organisms (Fig. 1B; Flemming et al., 2016; Flemming and Wingender, 2010;
142 Hansen et al., 2007). However, while the biofilm matrix grants increased survival, it also
143 enhances competition between inhabitants (Flemming et al., 2016; Rendueles and Ghigo,
144 2015). The growth and maturation of biofilms over time both result in the appearance of
145 environmental micro-niches and gradients within the matrix, with a centre generally
146 containing less dioxygen (O₂), fewer nutrients and more waste products (that is, more
147 stressful conditions; Rendueles and Ghigo, 2015). This environmental heterogeneity selects
148 for diversity within and among species through niche partitioning (Boles et al., 2004;
149 Cardinale, 2011; Flemming et al., 2016; Rendueles and Ghigo, 2015; Singer et al., 2010).
150 Biofilms can form complex communities including prokaryotes such as bacteria (e.g.
151 cyanobacteria) and archaea; as well as eukaryotes like plants, diatoms, fungi, other unicellular
152 eukaryotes, small metazoans (i.e. animals) and viruses (Fig. 1B; Battin et al., 2001; Costerton
153 et al., 1987; Geesey et al., 1978). Mechanisms of competition within biofilms are numerous
154 but can be divided in two groups: exploitative vs. interference competitions (Rendueles and
155 Ghigo, 2015). Exploitative competition refers to indirect interactions between organisms,
156 whereby one microorganism prevents access and/or limits the use of resources (e.g. O₂, iron)
157 by another microorganism. Interference competition corresponds to more direct mechanisms
158 such as predation; inhibition of growth, communication or biofilm colonisation; or induction
159 of biofilm dispersal (Rendueles and Ghigo, 2015). Colonisation and dispersal are important

160 biofilm processes (Battin et al., 2007; Flemming et al., 2016; McDougald et al., 2012). Many
161 microorganisms can live in both biofilm and planktonic lifestyles and can switch from one to
162 the other, the planktonic form being used to disseminate and colonise new environments,
163 while the biofilm form allows persistence. The processes of biofilm formation and, to a lesser
164 extent, detachment involve close communication between cells through density-dependent
165 signalling and gene-expression synchronisation, a phenomenon known as **quorum-sensing**
166 (Solano et al., 2014). Biofilm detachment can also be caused by external forces (allogenic
167 processes, e.g. shear stress) or by the death of deep layer cells (autogenic processes, Fig. 1B).
168 Such cooperation/competition and colonisation/dispersal mechanisms are essential to
169 understand processes of biofilm assembly. They will also determine whether biofilms might
170 act as a source or a sink for pathogens, a key question from a one health perspective
171 (Rendueles and Ghigo, 2015; Wingender and Flemming, 2011).

172 All in all, biofilms are very complex and heterogenous entities in space (gradients
173 creating microenvironments), time (maturation and succession of stages), composition (both
174 in species and EPS) and functions (Battin et al., 2016; Flemming et al., 2016; Flemming and
175 Wingender, 2010; Flemming and Wuertz, 2019). These properties are controlled by a broad
176 range of physical, biogeochemical and ecological factors, all of which are driven by
177 environmental processes (Fig. 2; Battin et al., 2016). Biofilms can be seen as mini-ecosystems
178 *per se* with their own community structure, productivity and resilience to external pressures,
179 which can reach **alternative stable states** (Flemming et al., 2016). For instance, one
180 study revealed that biofilm communities were able to recover faster to stable performance (i.e.
181 better resilience) after pH shock when diversity was initially high (indicating **functional**
182 **redundancy**) and competition between the two dominant genera (*Geobacter* and
183 *Methanobrevibacter*) was low (Feng et al., 2017). Therefore, concepts from ecological
184 theories (such as the metacommunity, ecological interactions and successions, niche
185 partitioning, **priority effects**), are essential to comprehend the biology and ecology of
186 biofilms (Battin et al., 2016, 2007; Besemer, 2015; Besemer et al., 2012). Only then will we
187 be able to fully understand the key roles that biofilms play in their environment, whether it be
188 a multicellular host or an ecosystem (Battin et al., 2016, 2003; Besemer, 2015; Besemer et al.,
189 2012; Peter et al., 2011). Here, we review the roles of biofilms communities and the ways
190 these might be impacted by global change. We distinguish between biofilms growing on non-
191 biological surfaces ('environmental biofilms') and biofilms growing on biological surfaces
192 (more particularly multicellular organisms, i.e. 'host-associated biofilms'). Making these
193 distinctions and using this terminology might reflect the biases from different research

194 perspectives, but these distinctions are helpful to demonstrate the different lenses that can be
195 used when considering the functional importance of biofilms.

196 **3. Importance of biofilms growing on biological interfaces**

197 **3.1. For Human and Animal Health**

198 Biofilms are problematic in human and veterinary medicine because bacteria or other
199 parasites can form biofilm on host tissues or implanted medical biomaterials, and cause
200 chronic infections (Clutterbuck et al., 2007; Costerton et al., 1999; Hall-Stoodley et al., 2004).
201 Pathogenic biofilms inherently allow their microorganisms to evade host immune defences
202 and resist antimicrobial agents (Mah & O'Toole, 2001). They can also act as a source of
203 pathogenic bacteria that can disperse and colonize new tissues (Fig. 3; Fleming & Rumbaugh,
204 2018). Notable diseases associated with biofilms include periodontitis, osteomyelitis, and
205 cystic fibrosis pneumonia in humans (Costerton et al., 1999), chronic ear infections in dogs,
206 and mastitis in cattle (Melchior et al., 2006; Moreira et al., 2012; Olson et al., 2002). Human
207 and other animal patients are particularly exposed to biofilm nosocomial infections when they
208 are in an immunodeficient state or in intensive care, and require the use of indwelling medical
209 devices (Lynch and Robertson, 2008). Biofilm-associated infections on animals can seriously
210 threaten human livelihoods and economy, for example by disrupting milk production (Fig. 3;
211 Melchior et al., 2006), and can pose a direct threat to human health since the involved
212 microorganisms are often zoonotic (e.g. *Staphylococcus aureus*; Clutterbuck et al., 2007;
213 Zambori et al., 2013). Since most antibiotics used in veterinary medicine are or were also
214 used in human medicine, animal biofilms largely contribute to the emergence of
215 antimicrobial-resistant pathogens, a major threat to public health (Roca et al., 2015).

216 However, there is increasing evidence that biofilms are also essential to human and
217 animal health, even though the literature is sometimes unclear as to whether biofilms are
218 considered *sensu stricto* or *sensu lato*. For example, dental biofilms are subject to much
219 research, both on oral health and disease (Marsh, 2006). True biofilms in healthy women have
220 been shown to exist in the urogenital tract and to act as an ecological barrier against
221 pathogens by preventing their adherence to cells (Chan et al., 1985; Domingue et al., 1991),
222 while more recent studies suggest that commensal (see **ecological interactions**) vaginal
223 lactobacilli prevent the growth of uropathogens like *Neisseria gonorrhoeae* by acidifying the
224 environment (Graver and Wade, 2011). The existence of biofilms *sensu stricto* on the walls of
225 the vertebrate digestive tract is more controversial. Their presence is confirmed in animal
226 species but consensus for humans is missing (de Vos, 2015; Lebeer et al., 2011; Palestrant et

227 al., 2004; Swidsinski et al., 2005; von Rosenvinge et al., 2013). Some medical researchers
228 detected true biofilms in the healthy human colon, arguing that direct observations were
229 previously precluded due to the difficulty of preserving the matrix after sampling (Bollinger
230 et al., 2007; Palestrant et al., 2004; Sonnenburg et al., 2004). However, the presence of
231 biofilms on food particles inside the digestive tracts was demonstrated multiples times, both
232 in humans and animals, suggesting that biofilms play significant digestive roles (Costerton et
233 al., 1987; Macfarlane and Dillon, 2007; Macfarlane and Macfarlane, 2006; Walker et al.,
234 2008).

235 If one considers biofilms in their broadest sense, their importance for human and
236 animal health becomes even more obvious. A substantial part of their microbiota lives
237 attached and/or incorporated within the mucus of the digestive tract, explaining why they
238 persist in such an ever-changing environment (Fig. 3; Martens et al., 2018; Sonnenburg et al.,
239 2004). There is increasing evidence of the roles of the gut microbiota in shaping the
240 behaviour, development, physiology and immunity of the host; conversely, **dysbiosis** is
241 implicated in the pathogenesis of multiple infectious and non-infectious diseases such as
242 obesity, irritable bowel disease, type-2 diabetes, certain cancers of the digestive tract,
243 cardiovascular diseases, and autoimmune syndromes and allergies (Clemente et al., 2012;
244 Ezenwa et al., 2012; Fung et al., 2017; Levy et al., 2017; Round and Mazmanian, 2009;
245 Sampson and Mazmanian, 2015). For instance, a study showed that early disruption by
246 antibiotics of the gut microbiome of Cuban tree frog tadpoles (*Osteopilus septentrionalis*) had
247 subsequent consequences for the resistance of the adult host to intestinal worm infection,
248 possibly due to an impairment of the immune system development in early life stages (Knutie
249 et al., 2017). The normal gut microbiome is also thought to act as a barrier against pathogens;
250 however, the specific mechanisms underlying this barrier effect remain in general little
251 understood (Bass et al., 2019; Reid et al., 2001). As an example, the role of two specific genes
252 (*ylaF* and *bssS*) in limiting the colonisation of a mouse commensal-*Escherichia coli* gut
253 biofilm by the two pathogens *Klebsiella pneumoniae* and diarrheagenic enteroaggregative *E.*
254 *coli* was demonstrated, but precise mechanisms are to be elucidated (Da Re et al., 2013). Skin
255 microbiomes (mucosomes) have also been shown to be relevant for host health (Fig. 3; Ross
256 et al., 2019), but have rarely been considered as biofilms while they are contained in skin
257 mucus in many vertebrates taxa such as fish and amphibians (Flemming and Wuertz, 2019;
258 Shephard, 1993; Toledo and Jared, 1993). Exceptions include the integument of marine
259 animals, such as corals and crustaceans, often colonised by biofilms *sensu stricto* which may

260 be detrimental by limiting light, gas, and nutrient availability and/or beneficial by interfering
261 with other predators and parasites (Qian et al., 2007; Wahl et al., 2012).

262 Biofilms roles in protecting against pathogens are of paramount importance since
263 emerging diseases pose a threat to humanity, biodiversity, and sustainability (Daszak et al.,
264 2000; Fisher et al., 2012). Most of these diseases originate in wildlife (Jones et al., 2008). As
265 an illustration, hard corals are keystone species for marine ecosystem health, on which a
266 substantial part of humanity relies for food (Moberg and Folke, 1999). Yet corals are
267 threatened by a range of factors, including climate change and emerging infectious diseases,
268 the former having a substantial impact on the latter (Harvell et al., 2002). Ritchie (2006)
269 found that the mucus from healthy Elkhorn coral (*Acropora palmata*) had a significantly
270 higher protective effect against certain pathogens than mucus sampled from heat-stressed
271 corals. There is also growing evidence that the occurrence, severity, and outcome of the
272 panzootic amphibian chytridiomycosis in susceptible amphibians are correlated with the
273 composition of skin microbial communities: a diverse skin microbiome being associated with
274 enhanced survival and dysbiosis with the disease status (Bernardo-Cravo et al., 2020).

275 Much remains unclear about the determinants (host genome, diet and lifestyle) and
276 functions of metazoan-associated biofilms (Heintz-Buschart and Wilmes, 2018; Huttenhower
277 et al., 2012). However, ancestral and modern human gut microbiomes were shown to differ
278 substantially (Blaser and Falkow, 2009; David et al., 2014; Sonnenburg and Sonnenburg,
279 2019). Homogenisation of habitat, sedentariness, industrialisation of food production,
280 increased use of antibiotics caused, amongst others, losses of commensal microbes and have
281 led to dysbiosis and a rise of non-infectious chronic diseases in human populations, such as
282 obesity. However, it remains difficult to disentangle the relative contributions of each factor
283 as all occurred more or less simultaneously with industrialisation (Blaser and Falkow, 2009;
284 David et al., 2014; Flandroy et al., 2018; Sonnenburg and Sonnenburg, 2019). Insights from
285 animal research confirmed this trend: captivity, pollution, land-use change, and climate
286 change, among other factors, generate dysbiosis and have negative effects on animal health,
287 with substantial implications for conservation (Bass et al., 2019; Trevelline et al., 2019; West
288 et al., 2019). For instance, warming of the environment led to gut dysbiosis and reduced
289 growth in *Ololygon perpusilla* tadpoles (Greenspan et al., 2020), while Xenobiotics (e.g.
290 lead) were also shown to alter the structure and functions of the human and animal gut
291 microbiome (Gao et al., 2017; Maurice et al., 2013).

3.2. For the Health of Plants and Other Macro-organisms

Terrestrial plants constitute the highest biomass on Earth (Bar-On et al., 2018), and their physiology and health fundamentally depend on the existence of symbiotic microbial communities which are mainly in the form of biofilms *sensu stricto* (Flemming and Wuertz, 2019; Morris and Monier, 2003; Rodriguez et al., 2019). Pathogenic biofilms exist in or on terrestrial plants (Morris and Monier, 2003; Rudrappa et al., 2008), but mostly biofilms are considered beneficial to the plant host (Compant et al., 2019; Rodriguez et al., 2019; Vandenkoornhuysen et al., 2015). Two biofilm communities are important for terrestrial plants: the leaf microbiome or phyllosphere (Vorholt, 2012), and the root microbiome or rhizosphere (Fig. 5; Lynch, 1994). There is strong evidence that the phyllosphere plays vital roles for terrestrial plants in terms of growth, functioning and fitness. These include resistance to biotic and abiotic stressors, as well as nutrient acquisition and cycling (Delmotte et al., 2009; Kembel et al., 2014). Although the concept of Home-field advantage (HFA) has long been considered to deal with soil microbial communities only, recent evidence shows that the phyllosphere plays significant roles in driving HFA effects through priority effects (Fanin et al., 2021). The aboveground phyllosphere would therefore impact ecosystem functioning by influencing nutrient dynamics and carbon cycling (Fanin et al., 2021). The rhizosphere is equally important for plant diversity, stability and interactions with other plants and organisms (e.g. fungi), thereby mediating biomass productivity. It is also important for enhancing tolerance and resilience to stress factors and affects root decomposition (Knief et al., 2012; Lynch, 1994; Zhou et al., 2020).

Plant biofilm communities offer the opportunity, through inoculation and enrichment with key microorganisms, to sustainably stimulate production and **bioremediation** (Compant et al., 2019). Emerging diseases of plants are also a serious threat to biodiversity, food security and the related economy (Anderson et al., 2004; Fisher et al., 2012) and both the healthy phyllosphere and rhizosphere have been shown to protect the host from pathogens, either through interference or by driving immunity (Castrillo et al., 2017; Pérez-de-Luque et al., 2017; Rudrappa et al., 2008). This provides opportunities for plant disease management (Poudel et al., 2016). As an example, *Bacillus subtilis* is widely used as a biocontrol agent and one study showed that it protected *Arabidopsis* sp. roots from the pathogen *Pseudomonas syringae* by forming biofilms and producing surfacin, an antimicrobial agent (Pal Bais et al., 2004). There has been growing awareness of the potentially adverse cascading effects that global change could have on ecosystems through changes in plant-associated biofilms (Delavaux et al., 2019; Vacher et al., 2016). A meta-analysis of 135 studies concluded that

326 elevated CO₂ had overall a positive influence on root-associated bacteria and fungi (Compant
327 et al., 2010). Regarding pollution, however, Jumpponen and Jones (2010) showed that
328 phyllosphere fungal communities of *Quercus macrocarpa* significantly differed between
329 urban and non-urban environments due to air pollution. The functional implications of such
330 shifts are still poorly understood, yet, the suppression of the phyllosphere microbiota by
331 antibiotic applications led to major shifts in the plant host metabolome (Gargallo-Garriga et
332 al., 2016). As to aquatic plants, they are also almost systematically covered by biofilms, with
333 similar beneficial and detrimental roles than for aquatic animals (Dang and Lovell, 2016;
334 Egan et al., 2013; Schiel and Foster, 2006; Wahl et al., 2012).

335 The ability of biofilms to protect plant or metazoan hosts against pathogens confirms
336 that the traditional disease triangle, often mentioned in disease ecology, should be extended to
337 a disease pyramid with the host-associated microbiome as a fourth vertex (Bernardo-Cravo et
338 al., 2020). The biofilm microbiomes form, with their host, a superorganism termed
339 holobiont (Carthey et al., 2020; Egan et al., 2013; Vandenkoornhuyse et al., 2015).
340 Increasingly, experts argue that conservation medicine and biology would benefit from a
341 paradigm shift to view not the host but the holobiont as a unit subject to selective pressures,
342 like those from global change (Carthey et al., 2020; Trevelline et al., 2019). However, many
343 knowledge gaps subsist (Table 1). For instance, it has yet to be determined whether biofilms
344 grow on and play significant roles for other multicellular organisms, such as macroscopic
345 fungi. It also remains difficult to predict how the interactive effects of e.g. climate change,
346 pollution, and land-use change will affect host health through their impacts on biofilms.
347 Consideration of host-associated biofilms in future research, but also in conservation practices
348 and developmental policies, will be essential to preserve host and ecosystem health in the
349 context of global change.

350 **4. Importance of biofilms growing on non-biological interfaces**

351 **4.1. For water and soil health**

352 Biofilms are the dominant mode of life in all habitats on Earth except open oceans
353 (Fig. 4; Battin et al., 2016; Flemming and Wuertz, 2019). Battin et al. (2016) qualified
354 biofilms as ‘the microbial skin’ of landscapes: a structuring, living and dynamic ecological
355 boundary where transition, contact, exchange but also separation of biotic or abiotic matter
356 occur. The soil biomass is dominated by bacteria and fungi mostly in the form of biofilms
357 (Cai et al., 2019; Flemming and Wuertz, 2019) and this soil microbiome is critical for
358 ecosystem functioning: it provides structure and stabilisation to friable soils and maintains

359 soil fertility by rendering key nutrients available to plants and other soil organisms (Ahmad et
360 al., 2017; Fierer, 2017). Freshwater microbiomes are constantly inoculated by soil
361 microorganisms via surface runoffs or subsurface flows (Fig. 4; Battin et al., 2016). Biofilms
362 develop on virtually all immersed solid–liquid interfaces such as rocks (epilithic biofilm or
363 epilithon), decaying wood (epixylic biofilm), sand grains (episammon), and mud sediments
364 (epipelon) (Fig. 4; Vadeboncoeur & Steinman, 2002). The terms periphyton and periphytic
365 biofilms cover all these meanings, including epiphyton (biofilms on plant organisms). Benthic
366 biofilms can be, in terms of biomass, the dominant component in many freshwater
367 ecosystems, especially when the water is shallow and/or has low turbidity (Besemer, 2015;
368 Vadeboncoeur and Steinman, 2002). Biofilms also develop on artificial substrates, such as
369 plastics and on other interfaces than solid–liquid like the neuston layer at aquatic-air interface
370 (i.e. pellicle) of marine and freshwater bodies (Flemming and Wuertz, 2019).

371 It is increasingly evident that biofilms regulate carbon fluxes in ecosystems, such as
372 boreal peatlands and streams (Battin et al., 2016; Wyatt et al., 2021). In these ecosystems, the
373 phototrophic community of biofilms ensures primary production, and their heterotrophic
374 community ensures the decomposition and cycling of carbon and other nutrients (Bartrons et
375 al., 2012; Battin et al., 2003; Besemer, 2015; Wu, 2017a). Biofilms also form a very
376 important trophic level, being the sometimes unique basis of the food webs (Hecky and
377 Hesslein, 1995; Rott et al., 2006; Vadeboncoeur and Steinman, 2002; Weitere et al., 2018).
378 This is particularly true for oligotrophic (e.g. alpine or boreal) ecosystems where conditions in
379 regard to temperature variability, wind, siltation and nutrient availability are extreme and
380 prevent vegetation development (Geesey et al., 1978; Lock et al., 1984; Rott et al., 2006;
381 Vadeboncoeur and Steinman, 2002). Biofilms provide the living environment for many
382 primary consumers and are grazed by both invertebrates and vertebrates such as tadpoles
383 (Füreder et al., 2003; Hecky and Hesslein, 1995; Rott et al., 2006). The trophic ecology of
384 biofilms and their consumers is of particular interest given their relevance in carbon cycling:
385 the absence of top-down controls by animals against biofilm herbivory was shown to increase
386 carbon dioxide emissions in boreal peatlands, highlighting the cascading effects of the loss of
387 biodiversity (Wyatt et al., 2021). Particularly, boreal ecosystems are expected to experience
388 changes in resource availability through climate change, shifting biofilm communities
389 towards heterotrophy under certain conditions (Myers et al., 2021).

390 Another important function of periphytic biofilms in fast-flowing waters is to
391 substantially increase water retention in quiescent areas (Battin et al., 2003). Increasing water
392 retention allows longer exchange of organic particles and enhances the ability of a system to

393 catch and retain usually-limiting nutrients such as phosphorus and nitrogen. In both lotic and
394 lentic freshwater ecosystems, biofilms act as a buffer for these nutrients and contribute
395 therefore to biogeochemical and ecological processes (Battin et al., 2003; Lu et al., 2016; Wu,
396 2017b). Biofilms also ensure the biostabilisation of sediments, thus limiting erosion
397 (Gerbersdorf and Wieprecht, 2015). Active denitrifying genes were also found in biofilms,
398 suggesting they also have an important role in regulating the global nitrogen cycle, which has
399 been substantially disrupted by the massive use of nitrogen-based fertilizers for food
400 production, emissions from fossil fuel combustion, and the increasing number of humans and
401 livestock (Gruber and Galloway, 2008; Vila-Costa et al., 2014). Such activities led to
402 eutrophication and acidification of aquatic ecosystems on a global scale; this is why nutrient
403 enrichment is considered a type of pollution which affect biofilms, but that biofilms can also
404 help mitigate. For example, periphyton biofilms were shown to decrease the levels of total
405 nitrogen and phosphorus in polluted rivers (Liu et al., 2016; Wu, 2017c). Nutrient enrichment
406 otherwise leads to algal blooms which can have devastating impacts on aquatic ecosystems by
407 producing harmful toxins, like microcystins, and depleting the water from resources such as
408 nutrients, dioxygen and light (Huisman et al., 2018). It was shown both *in-* and *ex-situ* that
409 periphyton biofilms made of diatoms and bacteria could control cyanobacterial blooms by i)
410 producing water-soluble allelochemicals (see *allelopathy*) that inhibit photosynthesis of
411 planktonic cyanobacteria; and ii) to a lesser extent, competing for key nutrients (Wu et al.,
412 2011). Periphyton biofilms could be used as a safe and practical bio-measure to limit algal
413 blooms and restore the health of hyper-eutrophic aquatic ecosystems, following provision of
414 substrates such as macrophytes (Liu et al., 2016; Wu et al., 2011). Periphyton biofilms were
415 also shown to remove the most common microcystin through adsorption and biodegradation,
416 with important implications for water quality, animal and human health (Wu et al., 2010).

417 Chemical pollution is also a major threat to global health since an ever-increasing
418 number of synthetic compounds are entering soils and water bodies, thereby decreasing water
419 quality even in remote mountain areas (Schmeller et al., 2018; Schwarzenbach et al., 2006).
420 Pollution can have an impact on biofilm growth and biomass, species composition, and on
421 biofilm functions (Boyero et al., 2019; Proia et al., 2013a; Sabater et al., 2007). However, to
422 date most studies have focused on one type of biofilm (on one single substrate) and one type
423 of contaminant exposed at one (often high) concentration in experimental settings (Bonnineau
424 et al., 2020). Little is known about the impacts that cocktails of compounds present at low
425 concentrations (i.e. natural conditions), have on the structure and functions of biofilms. Proia
426 et al. (2013a) showed that multi-toxin pollution led to significant change in biofilm structure

427 and functions *in situ*, with an increase in autotrophic biomass but a decrease in photosynthetic
428 efficiency. Analgesics and anti-inflammatories had major effects on biofilm responses,
429 notably by decreasing the ratio green algae/cyanobacteria (Proia et al., 2013a). Biofilms can
430 reduce freshwater and soil pollution by absorbing nutrients or synthetic pollutants into their
431 matrix, thus detoxifying the surrounding medium (Cardinale, 2011; Sabater et al., 2002).
432 Many toxicants can be captured and sometimes metabolised in environmental biofilms; these
433 include Persistent Organic Pollutants, but also other pesticides, heavy metals, human and
434 veterinary drugs, industrial effluents, nanomaterials, and plastics (Kohušová et al., 2011;
435 Sánchez-Pérez et al., 2013; Shabbir et al., 2020; Tang et al., 2018; Wu, 2017d, 2017e, 2017f).
436 Multispecies biofilms may generally be more efficient in remediation than single-species
437 biofilms due to synergistic interactions (Breugelmans et al., 2008).

438 Our review shows that biofilms buffer natural ecosystems against impacts of synthetic
439 molecules, nutrient pollution and cyanobacterial blooms (Edwards and Kjellerup, 2013; Wu,
440 2017g, 2017c). However, drivers of global change impact these important ecosystem services.
441 For instance, Cardinale (2011) showed that reduction of flow heterogeneity in stream
442 mesocosms and, subsequent absence of diverse niches, led to loss of biodiversity within
443 biofilms and lower removal of polluting nitrate. Similar results were found with glucose
444 (Singer et al., 2010). The diversity within biofilms reflects the diversity of their habitats,
445 therefore habitat loss and degradation might have great consequences on biofilms functions
446 within their ecosystems. In mesocosms experiments associated with long-term field studies,
447 Baulch et al. (2005) demonstrated that warming alone consistently led to increased bacterial
448 densities and metabolic rates. However, the extent of these changes differed depending on the
449 initial communities, with effects of substrate types (natural rocks vs. tiles) as well as biofilm
450 age (Baulch et al., 2005). Generally, *in situ* effects of global warming and other stressors are
451 difficult to predict because effects are not always additive, but rather synergistic or
452 antagonistic (Baulch et al., 2005; Romero et al., 2018). On river benthic biofilms, for
453 example, the effects of the herbicide triclosan were far worse when biofilms were also subject
454 to drought episodes (Proia et al., 2013b). More multiple-stressor research is needed to
455 understand how biofilm and the aquatic biota will respond to global change conditions.

456 Biofilms have short generation times, are ubiquitous and sensitive to toxicants and
457 other stressors (pH, salinity, nutrient pollution), meaning that their initial micro-ecosystem (in
458 terms of community structure, productivity and resilience) can definitely shift towards another
459 stable system (see theory of **Alternative stable systems**) more adapted to the new local
460 conditions (e.g. resistance to a pollutant; Burns and Ryder, 2001; Sabater et al., 2007; Wu,

461 2017h). This “ecological memory” made biofilms reliable indicators to assess and monitor
462 the health of aquatic ecosystems, with the development of many structurally-based or
463 functionally-based methods (Burns and Ryder, 2001; Sabater et al., 2007; Wu, 2017h). In a
464 recent survey monitoring pesticide occurrence in 54 Californian small streams, biofilms were
465 shown to contain, in average, four times as many current-use pesticides like pyrethroids as in
466 streambed sediments (Mahler et al., 2020). As biofilms adsorb toxicants and form the basis of
467 aquatic food webs, they make toxicants bioavailable to consumers, thus posing a risk to their
468 health (see part 4.3). In conclusion, not only are biofilms the sentinels of aquatic ecosystems,
469 they are also a critical control point to monitor and understand the fate of toxicants in food
470 webs (Bonnineau et al., 2020; Mahler et al., 2020).

471 **4.2. For the anthroposphere**

472 Biofilms also develop on man-made surfaces and pose difficulties in industrial settings
473 because they produce deleterious metabolites, clog filters and pipes, foul surfaces, and
474 corrode metals. Biofilms have wrought havoc in oil, gas and nuclear industries by degrading
475 pipelines and heat exchangers (Fig. 4; Beech & Gaylarde, 1999; Costerton et al., 1987). This
476 poses a direct threat to human health and economy, since biofouling and biocorrosion increase
477 the likelihood of industrial accidents and cost human societies trillions of US dollars in repair
478 or prevention (Beech and Gaylarde, 1999; Procópio, 2019). Environmental biofilms are also
479 major sources of concern and expense for maintaining good hygiene standards in clinical
480 settings, due to their inherent resistances to biocides and antimicrobial agents (Costerton et
481 al., 1999; Hall-Stoodley et al., 2004).

482 However, the ability of biofilms to adsorb, transform or degrade synthetic compounds
483 is also useful *ex situ*. Biofilms have been extensively employed as biotechnology to achieve
484 or increase bioremediation in a cost-effective manner, especially in the treatment of
485 wastewater (Fig. 6; Edwards and Kjellerup, 2013; Singh et al., 2006; Wu, 2017i). As an
486 example, periphyton biofilms were shown to effectively remove the azo-dye methyl orange
487 from textile industrial wastewater (Shabbir et al., 2017). Given the growing number of
488 humans and cities on Earth, these biofilm-based approaches may help mitigate pollution at a
489 global scale.

490 **4.3. For epidemiology**

491 Environmental biofilms can also affect the incidence, distribution, and determinants of
492 any health-related events, be it infectious or not. Toxin production has also been
493 acknowledged for biofilm cyanobacteria and can pose a threat to human and animal health

494 (Catherine et al., 2013; Mez et al., 1998; Wood et al., 2012). The environmental conditions
495 leading to toxin production are not yet fully understood. However, eutrophication, global
496 warming, water pollution, and human alteration of water flow were reported to increase the
497 frequency and intensity of these events (Catherine et al., 2013). Toxic blooms are often only
498 detected after mass mortalities occur, that is when toxin production is very high. The effects
499 of low exposure are unknown in natural conditions, although experimental findings have
500 shown effects of low concentrations of toxins on growth and reproduction on some
501 crustaceans, fish and amphibians (Dao et al., 2010; Oberemm et al., 1999).

502 Periphyton biofilms are an important food resource for many species, but biofilm
503 nutritional quality can vary depending on their composition, for example with regard to the
504 content of polyunsaturated fatty acids (omega-3). Omega-3 are essential for many species,
505 including humans, to ensure a correct metabolism and a competent immune system (Boëchat
506 et al., 2011; Guo et al., 2015; Hixson et al., 2015). Due to stressors, biofilm composition often
507 shifts from diatom-dominated towards cyanobacteria-dominated, with a decrease in omega-3
508 content (Crenier et al., 2019; Hixson et al., 2015; Leflaive et al., 2015). The implications of
509 such a reduced nutritional value have rarely been explored in practice for consumer health and
510 infectious disease susceptibility. However, Crenier *et al.* (2019) showed that a composition
511 shift could impact the growth and survival of a crustacean species. Similarly, the fact that
512 biofilms can adsorb pollutants can be deleterious for biofilm consumers, depending on the
513 fate of pollutants therein (Bonnineau et al., 2020). Microplastics are one example, being able
514 to impair periphyton growth and cause adverse health effects to tadpoles consuming these
515 contaminated biofilms (Boyero et al., 2019).

516 It is possible that these biofilm-related diseases (deficiencies in key nutrients, toxin or
517 chemical poisoning) influence the dynamics of wildlife and plant infectious diseases
518 (Supplementary Fig. S3). Rarely were microorganisms sympatric with pathogens considered
519 as biotic environmental drivers of infection and disease (but see Bernardo-Cravo et al., 2020;
520 Johnson et al., 2010; Schmeller et al., 2014; Supplementary Fig. S3). Yet, elimination of
521 human-pathogens from biofilms was demonstrated, following predation by biofilm-associated
522 protozoans or biofilm grazers (Fig. 1); inactivation by bacteriophage, or by antimicrobial
523 substances secreted by other microorganisms; or competition with other bacterial inhabitants
524 for nutrients and substrates (Chabaud et al., 2006; Langmark et al., 2005; Skraber et al., 2007;
525 Stevik et al., 2004). In contrast, some pathogens are known to survive, aggregate and even
526 replicate in or on biofilms (Langmark et al., 2005; Searcy et al., 2006; Skraber et al., 2007).
527 This is the case for numerous human water-borne pathogens, including viruses, bacteria and

528 protozoa (Hall-Stoodley and Stoodley, 2005; reviewed in Wingender and Flemming, 2011).
529 The behaviour and fate of pathogenic microorganisms within biofilms appear to depend on
530 the properties of the pathogen, those of the biofilm, the nature of the substrate, and
531 environmental parameters including those influencing desorption (Langmark et al., 2005;
532 Skrabber et al., 2007; Stott and Tanner, 2005). For instance, oocysts of *Cryptosporidium*
533 *parvum* appear to remain only at the surface of the biofilm without being incorporated and are
534 released back in the aquatic medium up to 40 days after adhesion (Searcy et al., 2006). Other
535 studies have shown that oocyst attachment could reach a saturated level in biofilms, varying
536 with biofilm roughness, which itself reflected environmentally-driven changes in the biofilm
537 community (Wolyniak et al., 2009; Wolyniak-DiCesare et al., 2012). How global change
538 impacts these processes is still not yet clarified. The role of environmental biofilms as
539 pathogen reservoirs has also been mostly studied for human and zoonotic pathogens, but very
540 rarely for those specific to animals and plants. Only two studies were found for non-human
541 pathogens: the first revealed that epilithic biofilms, including in high-elevation streams,
542 harboured the plant pathogen *P. syringae* (Morris et al., 2007); the other showed that tank
543 biofilms could serve as a reservoir of infection for *Mycobacterium chelonae*, a pathogen of
544 zebrafish (*Danio rerio* ; Chang et al., 2019).

545 Other biofilms may show the same or similar processes. The ocean surface microlayer
546 (Fig. 4), containing the neuston, covers up to 70% of the Earth's surface (Flemming and
547 Wuertz, 2019). It is three to five times richer in microorganisms than the rest of the water
548 column and likely drives important biogeochemical processes (Engel et al., 2017). As every
549 rising (aerosol) or incoming particle must pass through the surface microlayer, the neuston
550 participates in aerosol enrichment in microorganisms and plays a role in the diffusion of
551 pathogens (Aller et al., 2005). There is also a neuston community in freshwater bodies which
552 seems to play similar roles (Hervas and Casamayor, 2009). Other types of biofilms exist,
553 notably in the marine environment, such as marine snow (Simon et al., 2002) and biofilms
554 forming on microplastics (plastisphere) or other debris (Harrison et al., 2014; Kooi et al.,
555 2017). These have been shown to be relevant in epidemiology as they are involved in the
556 spread of pathogens (e.g. *Vibrio parahaemolyticus*; Kirstein et al., 2016) and harmful toxin-
557 producing species (e.g. dinoflagellates; Masó et al., 2003).

558 There are many knowledge gaps regarding environmental biofilms (Table 1). While it
559 is known that viruses (like other microorganisms) exist in biofilms and play huge roles in
560 aquatic ecosystems (Jacquet et al., 2010; Sutherland et al., 2004), there is still no
561 metabarcoding nor metabolomic methods for viruses that would allow a better understanding

562 of the virome within biofilms, especially in freshwater ecosystems (Marchesi and Ravel,
563 2015). Given the social and economic importance of the COVID-19 pandemic and other viral
564 epidemics, the study of the biofilm virome represents a promising avenue for research, as
565 biofilms can form reservoirs for enteric human-pathogenic viruses (Von Borowski and
566 Trentin, 2021; Wingender and Flemming, 2011). Current knowledge gaps on the interaction
567 between biofilms and pathogens stand in stark contrast to the major impediment to sustainable
568 development posed by increasingly numerous emerging infectious diseases. For instance,
569 *Batrachochytrium dendrobatidis*, causative agent of the panzootic amphibian
570 chytridiomycosis, is an aquatic chytrid fungus but its interactions with biofilms are unknown.
571 Doubtless, environmental biofilms are relevant in the ecology of some pathogens and the
572 disease pyramid concept provides an interesting framework to find and test hypotheses
573 (Supplementary Fig. S3). Besides, the biofilm life form promotes the exchange of antibiotic
574 resistance genes, but most research on antibiotic resistance focuses on planktonic bacteria in
575 man-made ecosystems (Balcázar et al., 2015; Hausner and Wuertz, 1999). Antimicrobial
576 resistance occurrence has increased in environmental microbiomes following inputs of human
577 and animal resistant microbes, and exposure to synthetic chemicals (Zhu and Penuelas, 2020).
578 Biofilms could be used as a preferred detection site of antibiotic resistant genes to assess the
579 risk of this threat to public health, especially in mountain ecosystems which provide water to
580 billions of human beings (Balcázar et al., 2015).

581 We note a sizeable knowledge gap regarding effects of biological stressors on
582 biofilms, such as human-mediated introduction of exotic species like fish who are often
583 introduced in usually fishless high-elevation lakes, with already known impacts on some taxa
584 (Miró et al., 2018). However, the effects of fish introduction on biofilms remain unknown.
585 Besides, pathogenic (potentially antimicrobial-resistant) or toxin-producing microorganisms
586 may be introduced with fish or livestock (Espunyes et al., 2021; Hunter and Thompson,
587 2005). Here, biofilms could serve as detection site for pathogen pollution as done in human
588 disease epidemiology to monitor environmental circulation of bacteria, protists and viruses
589 (Bauman et al., 2009; Wingender and Flemming, 2011). Finally, understanding the
590 interactions of all combined stressors on biofilms and the cascading effects on ecosystem will
591 arguably be the central challenge for future biofilm research (Chaumet et al., 2019a, 2019b;
592 Pesce et al., 2018; Romero et al., 2018).

593 **5. Conclusions**

594 It is essential that biofilm research is not neglected to identify, understand and mitigate
595 the interactive impacts of global environmental change on host and ecosystem health through
596 its effects on biofilms. To do so, we first need to capture the inherent natural variability –
597 relative to community structure, physical structure, and functions– of biofilm communities to
598 define what is a ‘normal’ biofilm in living organisms and ecosystems (Fig. 2). This will
599 require advances in both technological and theoretical frameworks. Advances in sequencing
600 technology and functional metagenomics will shed further light on biofilm composition and
601 functions. From a theoretical standpoint, we deem it useful to apply the analogy of health to
602 biofilms, as done for ecosystems (Rapport et al., 1998; Tett et al., 2013). The concept of a
603 ‘healthy’ biofilm, i.e. the state of a system that, given its location, its substrate, its age (stage
604 of succession) and the surrounding environmental conditions, contains an appropriate
605 functional diversity with all the expected organisms and their associated EPS. Such a biofilm
606 would be productive, resilient against external pressures, and capable of maintaining its
607 organisation and functions. One could argue that host-pathogenic biofilms or toxin-producing
608 periphytic biofilms could then be considered healthy because there are very productive.
609 However, these biofilms should be considered unhealthy because they usually appear as a
610 result of disturbances such as anthropogenic excessive loading (Wu, 2017j), over a relatively
611 short period of time, and are often dominated by a single species.

612 The theory of alternative stable states is central in the concept of biofilm health, as
613 when challenged beyond their resilience, biofilm transition to other stable state with different
614 community structure and functions. A healthy biofilm would reflect the stable state that is
615 optimal for, and indicative of, the good health of the larger system it inhabits, be it a
616 multicellular host or an entire ecosystem. For instance, unhealthy biofilms with less
617 biodiversity, such as host microbiome in dysbiosis, are less resilient and might be more
618 permeable to pathogen invasion (Feng et al., 2017; Keesing and Ostfeld, 2021). The concept
619 of healthy microbiomes has already been used in a medical context to designate a functional
620 microbiota associated with a healthy host (Clemente et al., 2012; Huttenhower et al., 2012).
621 We argue that the same should be applied to environmental biofilms and ecosystems,
622 respectively. Future research should produce more evidence supporting the diversity-stability
623 hypothesis in the diverse environments that biofilms inhabit. The concept of biofilm health
624 would help raise awareness about biofilm importance, promote scientific outreach, unify the

625 disciplinary silos of biofilm research in medical and environmental sciences and thus facilitate
626 transdisciplinary studies.

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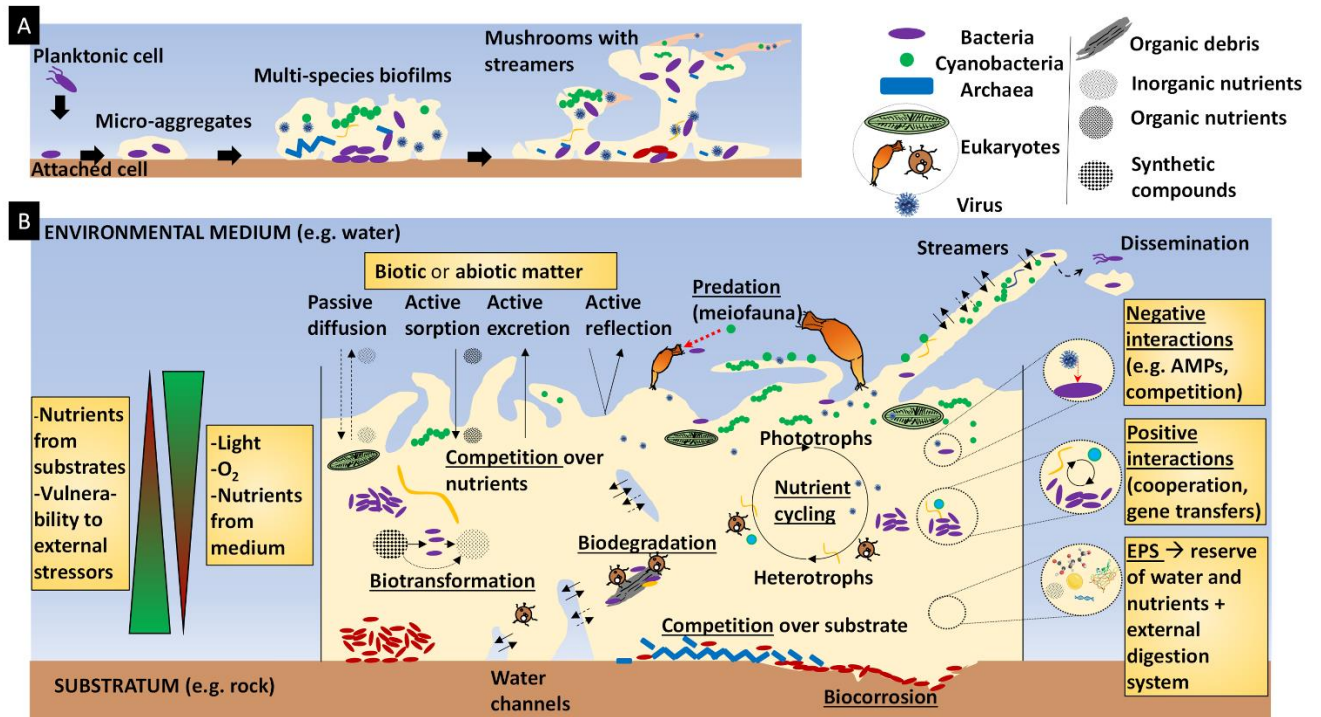
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Figures and Figures Legends



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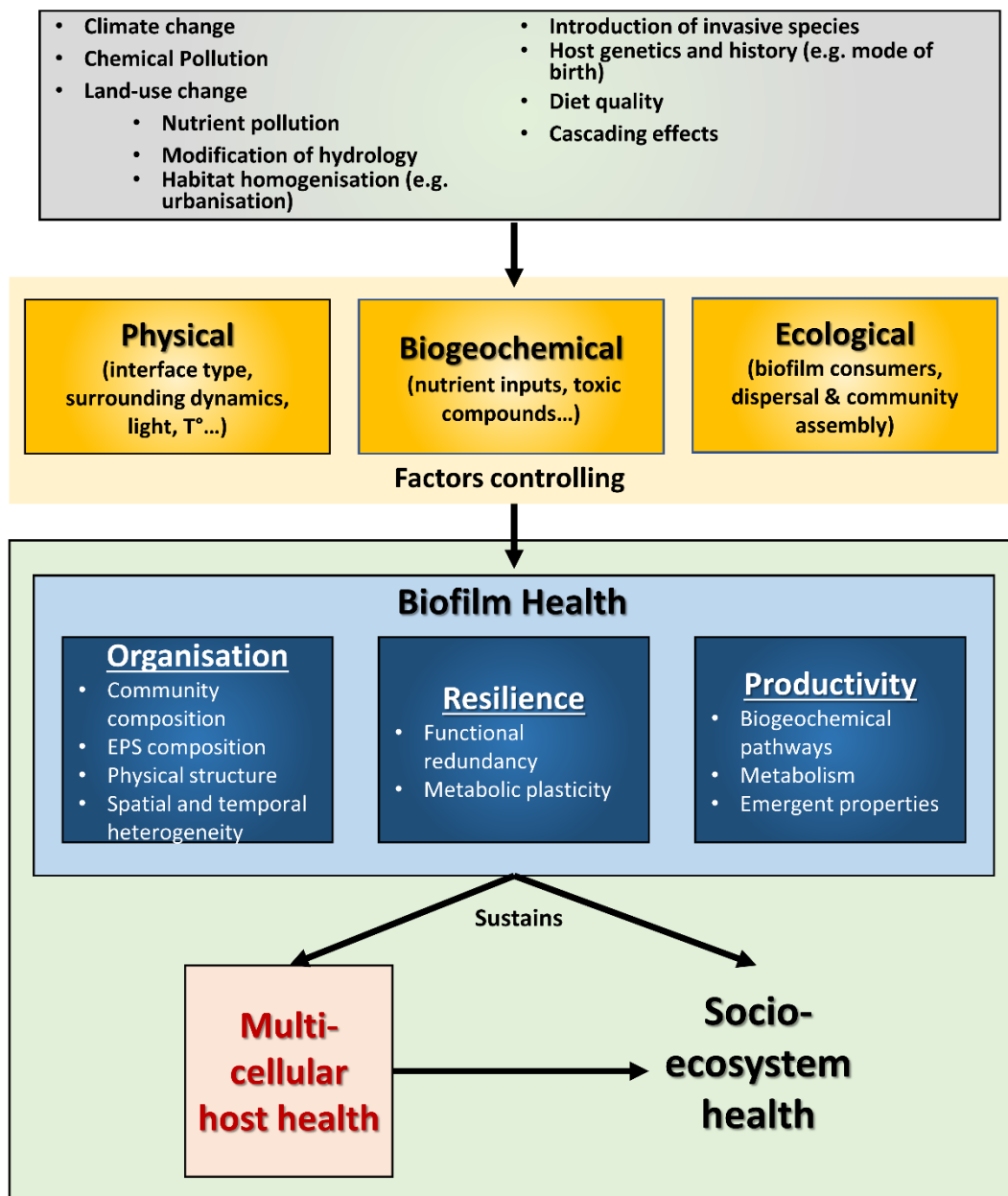
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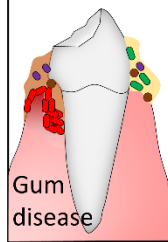
Figure 1: Structure and properties of biofilms. A: ecological successions from a single-attached cell to a mature biofilm, with multiple microbial species and streamers to intercept particles. B: Diversity and emergent properties conferred by the matrix within a mature biofilm. Abbreviations: AMP antimicrobial peptides, EPS extracellular polymeric substances.



1369 **Figure 2: Factors controlling biofilm health.** A: broad range of physical,
 1370 biogeochemical and ecological factors (yellow box) can drive biofilm health and have further
 1371 implications on the system where biofilms occur (green box; see also Fig. 3 and Fig. 4). These
 1372 factors are themselves impacted by drivers of global environmental change and/or host-related
 1373 factors (grey box).

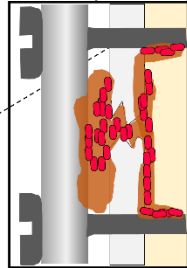
Pathogenic Biofilms

Teeth biofilm



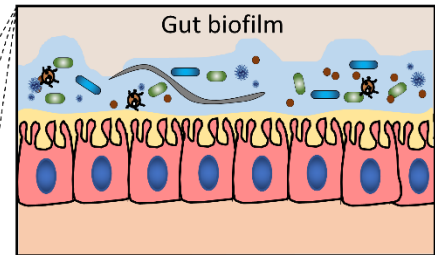
- **Disease pathogenesis** (gum disease, cystic fibrotic pneumonia in humans, skin wounds in animals, Pierce's disease in plants)
- **Increase resistance to host immune system and antimicrobial drugs** (general)
- **Delay healing processes** (general)
- **Source of complications** (e.g. osteomyelitis, septicaemia in animals and humans)

Complicated osteosynthesis



Beneficial Biofilms

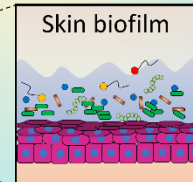
Gut biofilm



- **Contribute to normal physiology of the holobiont** (e.g. nutrient uptake for plants, animals and humans)

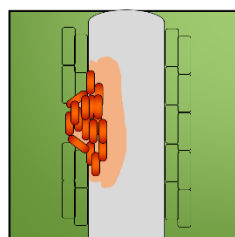
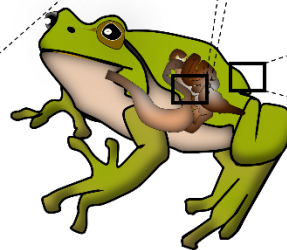
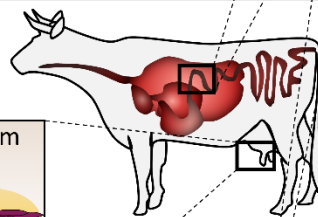
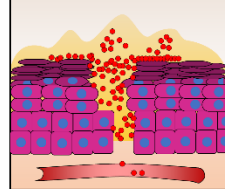
- **Shape host development, behaviour and immunity** (dysbiosis associated with non-communicable chronic diseases in humans)

Skin biofilm

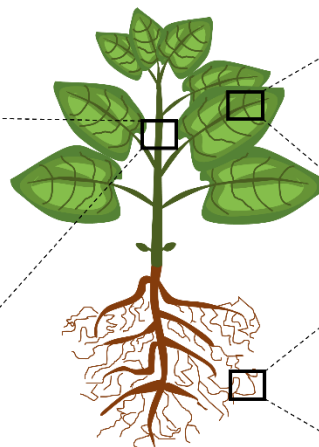


- **Barrier interfering with pathogens** (e.g. urogenital pathogens of humans, skin pathogens of amphibians)

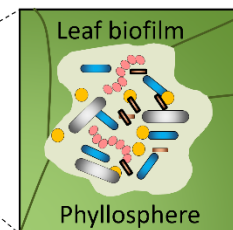
Mastitis biofilm



Xylem pathogenic biofilm

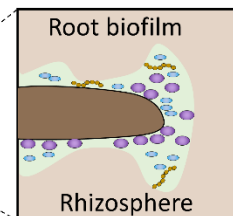


Leaf biofilm



Phyllosphere

Root biofilm



Rhizosphere

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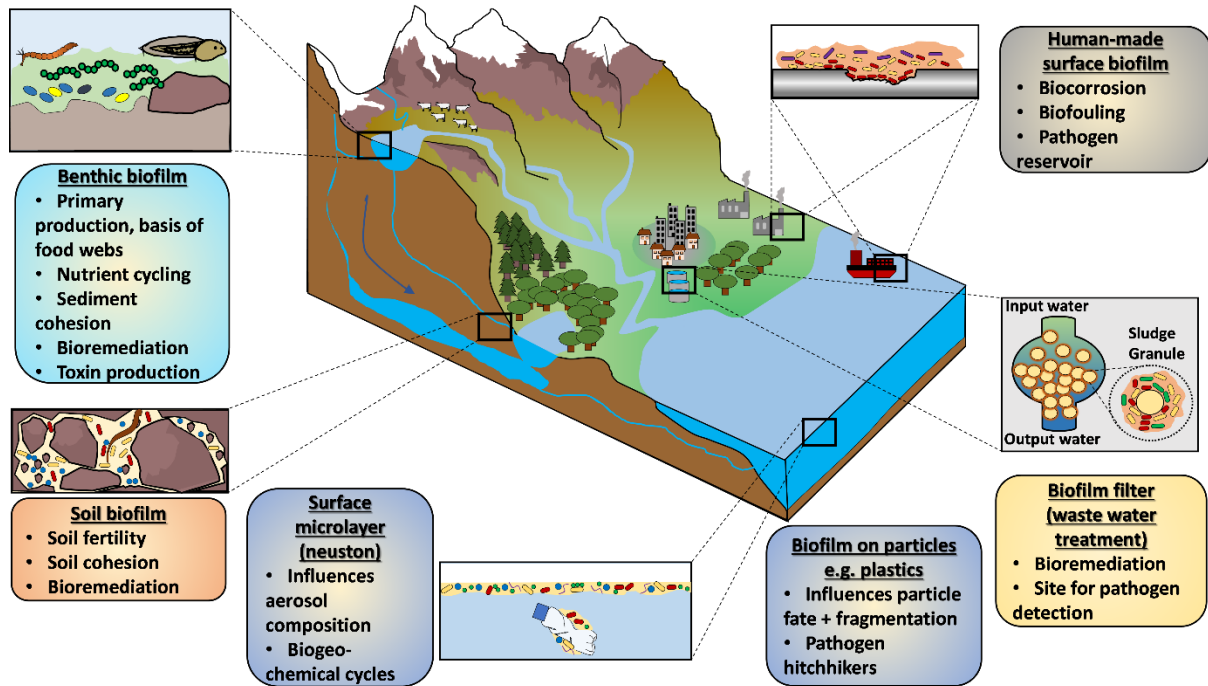
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Figure 3: Occurrence and roles of biofilms in disease (left) and health (right) for a human host, other animal hosts and a plant host. Biofilms are involved in the pathogenesis of some diseases including gum disease and secondary osteomyelitis (here a

1378 complication of a humeral fracture osteosynthesis is shown) in humans, mastitis in cattle, and
1379 Pierce's disease in plants. True beneficial biofilms occur in plants (rhizosphere and
1380 phyllosphere), around food particles ingested by humans and animals (e.g. ruminants), and on
1381 the lining of the urogenital tract of humans. Biofilms *sensu lato* occur on the gut of animals
1382 and humans and on the skin of animals with mucus (e.g. amphibians, fish).
1383

Preprint version



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1386

Figure 4: Occurrence and importance of biofilms at the scale of a landscape.

1387 Environmental biofilms can be seen as the microbial skin of landscapes as they are ubiquitous

1388 in many ecosystems including (i) terrestrial ecosystems: in freshwaters with benthic and

1389 hyporheic biofilms, the neuston of lakes, in the soil and the deep continental subsurface (not

1390 illustrated); (ii) marine ecosystems, with the ocean surface microlayer, on particles such as

1391 plastics and other debris, the upper sediment layer (not illustrated) and the deep oceanic

1392 subsurface (not illustrated); (iii) human-made surfaces like pipelines, boats or biofilm filters

1393 in waste water treatment.

1394

1395

1396

Table

1397

Table 1: Identified areas for further research on the structure and functions of biofilms

1398

growing on biological (host-associated biofilms) and on non-biological surfaces

1399

(environmental biofilms, see also Supplementary Figure S3)

Host-associated biofilms (growing on biological surfaces)	Determine whether biofilms <i>sensu stricto</i> on the lining of the human gut exist
	Determine whether biofilms exist on multicellular fungi
	Study the specific mechanisms underlying biofilm barrier effect against pathogens <i>in situ</i>
	Further study the determinants of host-associated biofilm community structure and functions
	Further study the mechanisms of resilience of host-associated biofilms that prevent dysbiosis
	Grasp the functional implications (using OMICS approaches) of the changes induced by climate change, habitat loss and pollution (taken separately and together) on animal and plant health through their effects on the associated biofilms
	Explore the virome of host-associated biofilms (taxonomy, abundance, functions and importance for host health)
Environmental biofilms (growing on non-biological surfaces)	Study the roles of environmental biofilms in infectious disease epidemiology, especially with regards to the fate of pathogens of global importance such as SARS-CoV-2 and <i>Batrachochytrium dendrobatidis</i>
	Study the roles of environmental biofilms in non-infectious disease epidemiology, e.g. in toxin production (including at low level), and explore the nutritional importance of biofilms for consumer health (in particular when biofilm transition from one state to another following a disturbance, e.g. diatom-dominated towards cyanobacteria-dominated)
	Impacts of cocktail of toxicants or nanoparticules at ecologically-relevant concentrations on <i>in situ</i> biofilm structure and functions
	Study the specific effects of stressors other than physical and chemical (biological stressors such as invasive species) on biofilms
	Pursue multiple stressor research (interactive effects of warming, change in hydrology, habitat loss and degradation, nutrient and chemical pollution, and other stressors) especially in regards with impact on functions and consequences for ecosystem health
	Explore the virome of environmental biofilms (taxonomy, abundance, biogeography, roles and importance of virus for ecosystem health), especially in freshwater ecosystems
	Further test the hypothesis of diversity-stability in various environments, exploring the mechanisms of resilience (existence of functional redundancy, niche partitioning, cooperation, competition) that prevent state shift (“dysbiosis”)
	Further study biofilms (structure and functions) other than the periphyton, such as the neuston, the plastisphere, marine snow and so on

1400 **Glossary**

1401 **Allelopathy:** biological phenomenon by which an organism produces one or more
1402 chemical substances able to influence (positively or negatively) the survival, growth, and/or
1403 reproduction of another organism.

1404 **Alternative stable states:** in ecology, this theory posits that ecosystems can exist
1405 under multiple "states" (sets of unique biotic and abiotic conditions) which are non-transitory,
1406 i.e. stable over ecologically-meaningful timescales. When disturbed beyond their capacity of
1407 resistance and resilience, ecosystems can transition from one state to another (termed a state
1408 shift) in which it will provide more or less, or altogether different ecosystem services.

1409 **Bioremediation:** method of sanitation used to treat any contaminated medium by
1410 stimulating microorganism-based biodegradation.

1411 **Diversity-stability theory:** this theory states that the more diverse a community is, the
1412 more stable (resilient) and productive it is, on the basis that more stable and productive
1413 communities can use their resources more efficiently than less diverse communities.

1414 **Dysbiosis:** term used to characterise any imbalance in a given microbiota (especially
1415 used for metazoan-associated microbiota), whether it be a change in species composition, of
1416 functions, or any disruption of its homeostasis. Dysbiosis is associated with a diseased state of
1417 the host.

1418 **Ecological interactions:** two-way interactions can be classified into 6 main types in
1419 ecology (0 denoting no effect, + a positive effect, - a negative effect): ++ mutualism (or
1420 cooperation); +/- commensalism; +/- antagonism (includes predation, grazing, parasitism,
1421 cannibalism and so on); -/- competition; -/0 amensalism; 0/0 neutralism. Note that facilitation
1422 (one species benefit from another species) includes mutualism, commensalism, and
1423 antagonism.

1424 **Ecological succession:** the process of change in the species structure of an ecological
1425 community over time. The whole series succession is sometimes called a sere, starting with
1426 one or few pioneering species and ending with a stable (i.e. in balance with the biotic and
1427 abiotic environment) or mature stage, called the climax (which could theoretically persist
1428 indefinitely if it was not for major disturbing events).

1429 **Emergent properties:** a system is said to have emergent properties if it displays
1430 properties that cannot be explained by its individual components. Not to be confused with the
1431 emergence of disease (sudden increase in incidence, distribution, and/or host range)

1432 **Functional redundancy:** synonym to functional equivalence, reflects that, in a
1433 community or ecosystem, multiple taxonomic groups (e.g. species) have similar functions
1434 (e.g. N fixation). Practically, it means that the loss of one taxon will not impact ecosystem
1435 functioning as long as other equivalent taxa can take over its roles.

1436 **Holobiont:** it is the assemblage of the host plus all its commensal and symbiotic
1437 species (living in or on the host). Synonym to superorganism. This term is increasingly used
1438 to denote that the host alone is not the unit subject to selective pressure, as multicellular
1439 organisms have likely always evolved with their microbiota, the latter being of paramount
1440 importance to maintain host health.

1441 **Home-Field Advantage:** this theory posits that microbial decomposers are specialised
1442 in using and colonising the substrates that they most frequently encounter, leading to the
1443 prediction that litter decomposition is faster in the close vicinity of the plant from which it
1444 originates, or in an area dominated by the same plant species.

1445 **Metacommunity:** A metacommunity is a set of local communities that are linked by
1446 the dispersal of multiple, potentially interacting, species. Patch dynamics (colonisation,
1447 competition), species sorting (due to environmental heterogeneity), source/sink dynamics
1448 (mass effect) and neutral perspective (stochastic demographic processes and limited dispersal
1449 abilities) are central frameworks of the metacommunity theory.

1450 **Niche partitioning (or differentiation):** process by which competing species use the
1451 environment differently in a way that helps them to coexist. For instance, different microbial
1452 decomposer or detritivore species will use different niches, with different food types or other
1453 resources. Resource partitioning is one of several complementarity mechanisms (facilitation is
1454 another one) leading to positive diversity effects.

1455 **Priority effect:** impact(s) that a particular species can have on community
1456 development due to prior arrival at a site. Can be inhibitive or facilitative.

1457 **Productivity (or vigor):** productivity is the rate at which energy is added to the bodies
1458 of organisms in the form of biomass. In ecosystems, there are two kinds of productivity:
1459 primary productivity, which refers to the energy (mostly solar) fixed by autotrophic
1460 communities into organic forms (chemical energy); and secondary productivity, which refers
1461 to heterotrophic communities which use the chemical energy produced by autotrophs.

1462 **Quorum-sensing:** Quorum sensing is the regulation of gene expression in response to
1463 fluctuations in cell-population density, mainly mediated by chemical signal molecules.

1464 **Resilience:** in ecology, resilience is the capacity of an ecosystem to respond to a
1465 perturbation or disturbance (e.g. fire, drought) by not only resisting damage but also being

1466 able to recover quickly to resume its original stable state. It is not a synonym of resistance, i.e.
1467 the property of ecosystem to remain unchanged (to resist changes) and stay in the stable state.
1468 However, resistance is a component of resilience.

1469 **Structure (or organisation):** the structure of a community or ecosystem refers to the
1470 compositions of taxa belonging to it, and their abundance. It relates to alpha-diversity, as a
1471 measure of species richness and evenness.

1472 **Xenobiotic:** a substance found within an organism that is not naturally produced or
1473 expected to be present within this organism.

1474

1475 **Acknowledgements**

1476 H.S. is supported by a Ph.D. scholarship from the French Ministry of Higher
1477 Education and Scientific Research. This work was further supported by the project People,
1478 Pollution, and Pathogens financed through the call “Mountains as Sentinels of Change” by the
1479 Belmont-Forum (ANR-15-MASC-0001 - P3, DFG-SCHM3059/6-1, NERC-1633948, NSFC-
1480 41661144004). D.S.S. holds the AXA Chair for Functional Mountain Ecology funded by the
1481 AXA Research Fund through the project GloMEc (Global Change in Mountain Ecosystems).
1482 Information on ongoing activities can be found on p3mountains.org. The authors declare no
1483 competing interest. Funding sources were not involved in the conceptualisation or writing of
1484 this manuscript.

1485 **Conflict of Interest statement**

1486 The authors declare no competing interest.

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