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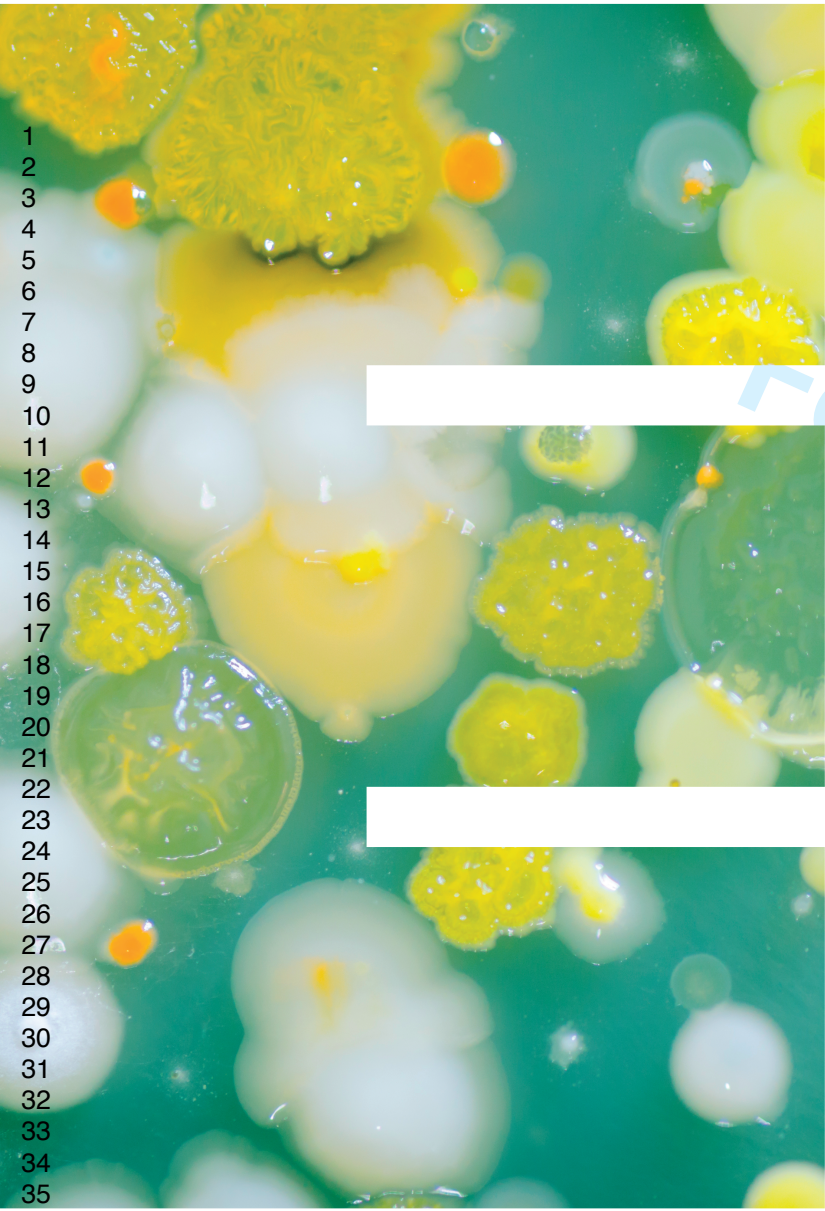
## 50 important research questions in microbial ecology

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**MICROBIAL**

**ECOLOGY**

**GROUP**

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3 **1 50 important research questions in microbial ecology**  
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38 Keywords:

39 environmental processes, evolutionary processes, functional diversity, host-microbiome interactions,  
40 priority setting, research agenda

41 **Abstract**

42 Microbial ecology provides insights into the ecological and evolutionary dynamics of microbial  
43 communities underpinning every ecosystem on Earth. Microbial communities can now be investigated  
44 in unprecedented detail, although there is still a wealth of open questions to be tackled. Here we  
45 identify 50 research questions of fundamental importance to the science or application of microbial  
46 ecology, with the intention of summarising the field and bringing focus to new research avenues.  
47 Questions are categorised into seven themes: Host-Microbiome Interactions; Health and Infectious  
48 Diseases; Human Health and Food Security; Microbial Ecology in a Changing World; Environmental  
49 Processes; Functional Diversity; and Evolutionary Processes. Many questions recognise that  
50 microbes provide an extraordinary array of functional diversity that can be harnessed to solve real-  
51 world problems. Our limited knowledge of spatial and temporal variation in microbial diversity and  
52 function is also reflected, as is the need to integrate micro- and macro-ecological concepts, and  
53 knowledge derived from studies with humans and diverse other organisms. Certain methods remain  
54 inadequate and currently limit progress in the field. Although not exhaustive, the questions presented  
55 are intended to stimulate discussion and provide focus for researchers, funders, and policy makers,  
56 informing the future research agenda in microbial ecology.

57

## 58 Introduction

59 In recent years, there has been an explosion in microbial ecological research, which is reflected in  
60 broad-scale research projects such as the Human Microbiome Project and the Earth Microbiome  
61 Project, as well as in the peer-reviewed literature (e.g. Boers *et al.*, 2016). Recent rapid technological  
62 advances, including next-generation sequencing, (meta)genomics, metabolomics,  
63 (meta)transcriptomics and (meta)proteomics, have vastly increased our ability to study microbial  
64 community complexity and function (Morris *et al.*, 2002; Hiraoka *et al.*, 2016). These provide  
65 unprecedented opportunities to assess genomic potential, gene regulation, expression and function *in*  
66 *situ* (Schneider *et al.*, 2012, Franzosa *et al.*, 2015), especially when combined with detailed knowledge  
67 of natural history and environmental parameters (Peay, 2014). Such techniques have been applied to  
68 a vast range of fields within the scope of 'microbial ecology' in order to better understand how  
69 microorganisms interact with and affect their environment, each other, and other organisms.

70 With an overwhelming and ever-growing number of potential and critical research avenues in  
71 microbial ecology, it is timely to identify major questions and research priorities that would progress  
72 the field. Here we present the results of a workshop hosted by the British Ecological Society's  
73 Microbial Ecology Special Interest Group in June 2016, which used a discussion and voting-based  
74 system to identify 50 research questions of importance to the field of microbial ecology. Similar  
75 exercises identifying important research questions have been conducted in conservation (Sutherland  
76 *et al.*, 2009, Dicks *et al.* 2012), pure ecology (Sutherland *et al.*, 2013a), marine biodiversity (Parsons  
77 *et al.*, 2014), sustainability (Dicks *et al.*, 2013; Jones *et al.* 2014), and non-ecological subjects  
78 including UK poverty (Sutherland *et al.*, 2013b). These papers have been widely accessed and are  
79 directly applicable to the development of policy, as highlighted by Jones *et al.* (2014).

## 82 Methods

### 83 *Participants*

84 The methods used here were based broadly on those presented in Sutherland *et al.* (2011). A one-day  
85 workshop was held by the British Ecological Society's Microbial Ecology Special Interest Group at the  
86 University of Salford (UK) in June 2016. Invitations to attend the meeting were distributed via the



1  
2  
3 87 British Ecological Society's membership mailing list and through social media (Twitter and Facebook).  
4  
5 88 In total, 34 participants from 20 institutions attended and contributed to the development of the 50  
6  
7 89 questions listed below, with the majority listed as authors on this paper.  
8

9 90

10  
11 91 *Questions*

12  
13 92 Prior to the workshop, attendees were asked to submit questions via an online form that they thought  
14  
15 93 most closely met the following brief:

16  
17  
18 94 "We are aiming to identify 50 questions that, if answered, will make a considerable  
19  
20 95 difference to the use of microbial ecology by practitioners and policy makers, or to  
21  
22 96 the fundamentals of the field of microbial ecology. These should be questions that  
23  
24 97 are unanswered, could be answered, and could be tackled by a research  
25  
26 98 programme. This is expected to set the agenda for future research in the field of  
27  
28 99 microbial ecology."  
29

30 100

31  
32 101 A total of 244 questions were submitted by attendees (see Supplementary Information), and assigned  
33  
34 102 (by R.E. Antwis and S.M. Griffiths) to the following themes;

- 35  
36 103 1) *Host-Microbiome Interactions*;
- 37  
38 104 2) *Health and Infectious Diseases*;
- 39  
40 105 3) *Human Health and Food Security*;
- 41  
42 106 4) *Microbial Ecology in a Changing World*;
- 43  
44  
45 107 5) *Environmental Processes*;
- 46  
47 108 6) *Functional Diversity*;
- 48  
49 109 7) *Evolutionary Processes*.

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54  
55 111 An additional eighth theme named '*Society and Policy*' was created to capture a number of questions  
56  
57 112 that were generally applicable across the biological sciences, as well as a number of questions  
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3 113 specific to the field of microbial ecology which could not necessarily be addressed through laboratory  
4  
5 114 based microbial ecology research, *per se*.

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7 115

8  
9 116 *Question selection process*

10  
11 117 Prior to the workshop, participants were asked to identify the top ~20% of questions in each theme  
12  
13 118 that most closely aligned with the brief (selection of 5-11 questions from a total of 26-57 questions per  
14  
15 119 theme via online form; Supplementary Information). Participants were asked to consider all questions  
16  
17 120 within a theme and to select questions based on the theme's context and the brief for the workshop.  
18  
19 121 Some questions were included in more than one theme to encourage discussion and to increase the  
20  
21 122 likelihood that pertinent questions remained in the selection process. Questions were then ranked  
22  
23 123 according to the number of online votes they received, and this formed the material for the workshop.

24  
25 124 Three sets of parallel sessions were run at the workshop, with participants free to select which  
26  
27 125 theme sessions they attended. Questions were discussed in order of lowest ranking to highest, with  
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29 126 duplicates removed and questions reworded as necessary. For each theme, a final set of 'gold' (~15%  
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31 127 of questions, total of 47 questions across all themes) and 'silver' questions (~10% of questions, total of  
32  
33 128 29 questions) were identified. Where necessary, a show of hands was used to ensure the democratic  
34  
35 129 process was upheld.

36  
37 130 A final plenary session was held in which all gold and silver questions were discussed. For  
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39 131 gold questions, duplicates among categories were removed and questions reworded to reflect the  
40  
41 132 discussion in the room, resulting in 43 gold questions. A similar process was then completed for silver  
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43 133 questions, and a show of hands used to vote for seven questions that could be elevated to gold status  
44  
45 134 to form the final set of 50 questions.

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47 135

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49 136 *Limitations*

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51 137 All but four participants were from British universities, although there were representatives from a  
52  
53 138 range of nationalities and research areas. The manner in which this paper was developed (*i.e.* through  
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55 139 a physical workshop and via the British Ecological Society) means that, without a substantial travel  
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57 140 budget, a bias towards UK institutions was inevitable. However, many participants have worked on, or

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2  
3 141 currently collaborate in, research projects on non-UK ecosystems and species, and therefore the  
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5 142 questions proposed are drawn from considerable knowledge and experience of the field  
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7 143 internationally. Additionally, although most individuals were from academic institutions, many  
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9 144 individuals had previous or on-going collaborations with industrial partners and governmental/non-  
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11 145 governmental organisations.

12  
13 146

## 14 147 **Results**

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17 148 The following 50 questions are presented by theme, and are not ordered according to relevance or  
18  
19 149 importance. Due to the nature of the process, some questions may appear similar across themes, but  
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21 150 within the context of each theme can take on a different meaning. Some questions may relate to  
22  
23 151 research areas that are already somewhat active, and these serve to highlight the importance of and  
24  
25 152 encourage further work in these areas. Some of these questions apply across multiple biomes and  
26  
27 153 ecosystems, and can be considered in the context of multiple host organisms and across varying  
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29 154 temporal and spatial scales.

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## 32 33 156 ***Host-Microbiome Interactions***

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35 157 Host-microbiome interactions determine many host life history traits such as behaviour, reproduction,  
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37 158 physiological processes, and disease susceptibility (Archie and Theis, 2011; Willing *et al.*, 2011; Koch  
38  
39 159 & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.*, 2016). Increasingly, we are discovering  
40  
41 160 that host-microbiome interactions produce complex and dynamic communities that fluctuate in  
42  
43 161 compositional abundance correlated with factors as diverse as host genotype, developmental stage,  
44  
45 162 diet, and temporal changes, among others (e.g. Spor *et al.*, 2011). Even in otherwise well studied  
46  
47 163 organisms, very little is known about the consequences of microbiome variation for host processes,  
48  
49 164 particularly across different spatial and temporal scales. Considerations of host microbiomes are also  
50  
51 165 likely important for global issues, such as the efficacy of conservation efforts including species  
52  
53 166 reintroduction programmes (reviewed in Redford *et al.*, 2012; McFall-Ngai, 2015). Additionally,  
54  
55 167 interactions between native and non-native species are correlated with transmission of microbiota,  
56  
57 168 often determined by relatedness or diet type (Ley *et al.*, 2008), and the microbiome plays a key role in  
58  
59 169 the control and competence of insect crop pests and vectors of disease (reviewed in Weiss & Aksoy,  
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3 170 2011). The following questions aim to address the shortfall in our understanding of the interactions  
4  
5 171 between microbiomes and their human and non-human hosts.  
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9 173 1. What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe  
10  
11 174 interactions?

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13 175 2. What are the relative contributions of host-associated and environmental factors in determining host  
14  
15 176 microbial community composition?

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17 177 3. How do microbial communities function to affect the phenotype of the host?

18  
19 178 4. Can compositional or evolutionary changes in microbiomes help hosts adapt to environmental  
20  
21 179 change within the lifetime of the host?

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23 180 5. What is the role of the microbiota in host speciation processes?

24  
25 181 6. How can the associated microbiota be effectively included in risk assessments of Invasive Non-  
26  
27 182 Native Species?

28  
29 183 7. How does the microbiome of captive animals affect the success of reintroduction programmes?

30  
31 184 8. How can a 'systems biology' approach improve our understanding of host-microbe interactions?  
32

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37 186 ***Health and Infectious Diseases***

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39 187 The last 50 years have seen the emergence of several hypervirulent wildlife pathogens in animals  
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41 188 (e.g. Tasmanian devil face tumour disease, avian malaria, amphibian chytridiomycosis; reviewed in  
42

43 189 Tompkins *et al.*, 2015) and plants (e.g. sudden oak and larch death, ash dieback; Pautasso *et al.*,  
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45 190 2015). Although the role of microorganisms as pathogens is well known, the importance of host-  
46  
47 191 associated microbiomes in regulating disease susceptibility is becoming more apparent (Koch &  
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49 192 Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.*, 2016). A major outstanding research goal is  
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51 193 to understand how within-host interactions among microbes and invading pathogens may shape  
52

53 194 patterns of infection intensity and disease progression (see also *Evolutionary Processes*). Several  
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55 195 studies have sought to determine how manipulation of host microbiomes may ameliorate the spread  
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57 196 and impact of such diseases (e.g. Rebollar *et al.*, 2016).  
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3 197 While for many disease states the paradigm holds true that one microorganism causes one  
4 198 disease, polymicrobial infections are becoming more apparent through metagenomic and  
5 199 metatranscriptomic sequencing of disease-associated microbial communities (Gilbert *et al.*, 2016).  
6  
7 200 Consequently, the 'pathobiome' concept, where a disease state is influenced by complex interactions  
8  
9 201 between commensal and pathogenic microorganisms, presents new challenges for applying Koch's  
10  
11 202 postulates to diseases arising from polymicrobial interactions (Vayssier-Taussat *et al.*, 2014), such as  
12  
13 203 black band disease (BBD) in corals (Sato *et al.*, 2016) and olive knot disease (Buonaurio *et al.*, 2015).

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16 204 In this theme we have identified research questions relating to the microbial ecology of  
17  
18 205 infectious diseases and host health. Although much can be learnt from the comparatively high number  
19  
20 206 of studies in the human and biomedical literature (*e.g.* using network approaches in epidemiology), the  
21  
22 207 questions selected in this theme predominantly relate to non-human animals and plants, as humans  
23  
24 208 are covered later (*'Human Health and Food Security'*).

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28 210 9. How can we better track the source and dispersal of particular microorganisms in real time?

29  
30 211 10. Many microorganisms are unculturable, and many microbiome studies reveal that diseases are  
31  
32 212 polymicrobial; how can we re-evaluate Koch's postulates in this context?

33  
34 213 11. Which factors trigger 'covert' infections to become 'overt', impacting host health?

35  
36 214 12. At the population level, how is the burden and shedding intensity of intracellular microbes affected  
37  
38 215 by co-infection by extracellular parasites?

39  
40 216 13. What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms  
41  
42 217 of their survival and spread?

43  
44 218 14. How can network theory best be used to predict and manage infectious disease outbreaks in  
45  
46 219 animals and plants?

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48 220 15. Can microbiomes of wildlife (plants and animals) be used or manipulated to enhance health and/or  
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50 221 disease resistance?

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56 223 ***Human Health and Food Security***

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3 224 With the human population due to exceed eight billion by 2024, food security and human health are  
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5 225 high on political and scientific agendas. The human microbiome has been the focus of intense  
6  
7 226 research efforts in recent years, (e.g. Walter & Ley, 2011; Spor *et al.*, 2011; Mueller *et al.*, 2012),  
8  
9 227 because gut symbionts shape the immune response (Round *et al.*, 2009), and diversity fluctuates  
10  
11 228 through chronic conditions and infectious diseases including diabetes, obesity (Serino *et al.* 2016;  
12  
13 229 Baothman *et al.*, 2016; Ridaura *et al.*, 2013), asthma (Smits *et al.* 2016), and HIV (Lozupone *et al.*,  
14  
15 230 2013). Improving our understanding of the core human microbiome and individual variation will  
16  
17 231 underpin pharmomicrobiomics, enabling development of novel therapeutic treatments and, ultimately,  
18  
19 232 personalised medicine (e.g. Ubeda *et al.*, 2013).

20  
21 233 Antibiotic resistance resulting from selective pressures generated by the use and misuse of  
22  
23 234 antibiotics is a global threat to public health (Levy, 1997; Tam *et al.*, 2012). The volume of antibiotics  
24  
25 235 used in agriculture now exceeds the amount used in human medicine in many countries (WHO, 2011).  
26  
27 236 Antibiotics are still widely used in livestock for prophylaxis and growth promotion, often at sub-  
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29 237 therapeutic concentrations, exacerbating resistance (Krishnasamy *et al.*, 2015). The impact of the  
30  
31 238 leaching of antibiotics into the natural environment and subsequent impacts on natural microbial  
32  
33 239 communities remains poorly characterised (Franklin *et al.*, 2016). Current practices of growing high-  
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35 240 intensity monoculture crops have a negative impact on the microbial biodiversity of soils through a  
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37 241 combination of tillage, subsequent erosion and chemical applications (Helgason *et al.*, 1998; Jacobsen  
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39 242 and Hjelmsø, 2014; Zuber and Villamil, 2016), which imposes selection pressures on pathogenic  
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41 243 microbes, fungal symbiotic partners and plant growth promoting bacteria (Chapparo *et al.*, 2012;  
42  
43 244 Hartmann *et al.*, 2015). Thus, there is a need to maintain and enhance microbial populations of crop  
44  
45 245 ecosystems, especially in light of antibiotic resistance (Ellouze *et al.*, 2014). As antibiotic resistance  
46  
47 246 increases, along with our concern about potential impact on both human and animal health, there is an  
48  
49 247 increasing drive to find new forms of antibiotics.

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51 248 Though the remit for this section is relatively broad, the questions focus on two central  
52  
53 249 themes: i) studying the human microbiome to improve the treatment of disease, including the  
54  
55 250 development of personalized medicine and novel antibiotics; and ii) understanding how *current*  
56  
57 251 antibiotic regimes and farming practices may negatively impact the diversity of the environmental  
58  
59 252 microbiome and food production capacity.  
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3 254 16. How can human microbiome studies improve personalised medicine?  
4  
5 255 17. What ecological principles can be applied in the search for new antibiotics and alternatives?  
6  
7 256 18. What are the main determinants of waterborne infection outbreaks, and what is the best strategy  
8  
9 257 to control these in water distribution systems?  
10  
11 258 19. What are the consequences of antibiotic and pharmaceutical use in human medicine on microbial  
12  
13 259 communities in freshwater and soil environments?  
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16 260 20. To what extent are microbial species distributions influenced by climate, and what are the  
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18 261 consequences for food security and human health?  
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20 262 21. How much microbial diversity in the soil has been lost through monoculture and what is the  
21  
22 263 importance of this?  
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24 264 22. Intensive farming may involve high levels of agrochemicals and broad-spectrum antibiotic usage -  
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26 265 what will be the long-term effects on microbial communities?  
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28 266 23. How best can we harness microbial communities to enhance food production?  
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31 267

32  
33 268 ***Microbial Ecology in a Changing World***

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35 269 Global changes resulting from human activity impact almost every habitat on earth. It is imperative that  
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37 270 we focus efforts on understanding the impacts of human activities such as climate change,  
38  
39 271 urbanisation, agriculture, and industrial processes on microbial communities, ecosystem functioning  
40  
41 272 equilibrium, and host health. Microbial populations have a tremendous capacity to adapt to changes in  
42  
43 273 their abiotic environment, yet the functional implications of these transitions in microbial ecology are  
44  
45 274 still poorly understood and characterised (Bissett *et al.*, 2013), and the role of microbes in mediating  
46  
47 275 the response of larger organisms to change is equally understudied. Global environmental changes  
48  
49 276 (GECs) are complex and multifaceted. Human activities such as urbanisation, land-use change and  
50  
51 277 introduction of invasive species have played a role in shifting global ecosystems via desertification,  
52  
53 278 climate change and habitat degradation. Although such changes have been quantified in aquatic and  
54  
55 279 terrestrial habitats (*e.g.* Haberl *et al.*, 2007; Halpern *et al.*, 2008), their effects on microbial  
56  
57 280 communities and impacts on ecosystem function are often hindered by a lack of characterisation of  
58  
59 281 communities, or limited understanding of microbial functional traits. Shifts in basic nutrients and gases  
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3 282 such as CO<sub>2</sub>, along with temperature fluctuations and water availability, greatly influence the  
4  
5 283 distribution and behaviour of species (Tylianakis *et al.*, 2008). GECs can alter host fitness or  
6  
7 284 ecosystem functioning (Shay *et al.*, 2015; Webster *et al.* 2016) and are likely to occur in combination.  
8  
9 285 While there is a great deal of research into the effects of each of these on microbial communities  
10  
11 286 (Schimel *et al.*, 2007; Shurin *et al.*, 2012; Lloret *et al.*, 2014), literature considering the effect of  
12  
13 287 multiple GECs is sparser, and these have complicated and often unpredictable consequences when  
14  
15 288 combined (although see Hutchins *et al.*, 2009; Ryalls *et al.*, 2013). In this section, we consider how  
16  
17 289 human activities directly and indirectly influence the microbial world. Where applicable, these  
18  
19 290 questions can be considered across multiple biomes and ecosystems, with reference to resulting  
20  
21 291 trophic cascades, in addition to the impacts on multiple biogeochemical processes. We also consider  
22  
23 292 how microbes can be used as a tool for mitigation or bioremediation of human-induced environmental  
24  
25 293 changes, and the ways in which microbes can be included in current evaluations of global change.

26  
27  
28 294

29 295 24. How can we integrate microbial communities into models of global change?

30 296 25. Will ocean acidification, temperature increases and rising sea levels lead to changes in microbial  
31  
32 297 diversity or function, and what will the cascading effects of this be?

33  
34 298 26. How do human activities, such as oil and gas drilling, influence the sub-surface microbiome(s)?

35  
36 299 27. How will increasing urbanisation affect environmental and host-associated microbial communities?

37  
38 300 28. How resilient are different microbial functional groups to ecosystem disturbance?

39  
40 301 29. Can we manipulate microbial succession in species-poor soils to encourage repopulation by flora  
41  
42 302 and fauna?

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#### 46 47 48 304 **Environmental Processes**

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50 305 Microbes play a fundamental role in environmental processes and ecosystem services, including  
51  
52 306 nutrient cycling and organic matter decomposition (Chin *et al.* 2016; Creamer *et al.*, 2015; Weider *et*  
53  
54 307 *al.*, 2013), bioremediation of contaminated habitats or waste systems (Haritash & Kaushik, 2009; Oller  
55  
56 308 *et al.*, 2011), and influencing greenhouse gas emissions (Singh *et al.*, 2010; Bragazza *et al.*, 2013; Hu  
57  
58 309 *et al.*, 2015). The ability to harness these processes has great potential for societal and environmental



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3 310 applications, particularly in extremophiles, which frequently reveal metabolic capabilities and  
4  
5 311 evolutionary solutions not witnessed elsewhere in the microbial world (Coker et al. 2016). However, it  
6  
7 312 is rarely possible to directly link the presence of a specific microbial taxon to a particular ecological  
8  
9 313 process. Other methodological challenges include establishing the relative importance of biotic and  
10  
11 314 abiotic factors in microbial ecosystem function, and determining the appropriate spatial and temporal  
12  
13 315 scale necessary to discriminate links between microbiota and their ecological functions (Bissett *et al.*,  
14  
15 316 2013). Concurrently, a deeper understanding is required of human-induced impacts on the global  
16  
17 317 microbiome through urbanisation, habitat degradation, climate change, and the introduction of invasive  
18  
19 318 species, amongst others.

20 319

21  
22 320 30. How do we successfully establish microbial communities used in bioremediation?  
23

24  
25 321 31. How important is the rare microbiome in ecosystem function, and how does this change with  
26  
27 322 stochastic events?

28  
29 323 32. To what extent is microbial community diversity and function resilient to short- and long-term  
30  
31 324 perturbations?

32  
33 325 33. What is the importance of spatial and temporal variation in microbial community structure and  
34  
35 326 function to key environmental processes and geochemical cycles?

36  
37 327 34. How can we accurately measure microbial biomass in a reproducible manner?  
38

39  
40 328 35. Which mechanisms do extremophiles use for survival and how can they be exploited?  
41

42 329

### 43 44 330 **Functional Diversity**

45  
46 331 Ecologists are increasingly turning their attention to classifying species based on their activity  
47  
48 332 (function) within an ecosystem, rather than their genotype (Crowther *et al.*, 2014). This is particularly  
49  
50 333 relevant for microbial ecology, in which species are hard to define, horizontal gene transfer is rife, and  
51  
52 334 taxonomy is often blurred. Understanding how membership within complex and dynamic microbial  
53  
54 335 communities relates to the function of that community is one of the key challenges facing microbial  
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56 336 ecology (Widder *et al.*, 2016). This is true across a vast range of spatial scales, from microbial dyads  
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58 337 to the gut of a *Drosophila* fly, to ancient trees and their associated ecosystems, right through to global

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3 338 biogeochemical processes. There is an urgent need to understand how the genome of a microbial  
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5 339 community (and in some cases, its host) relates to metabolic capacities. Conversely, there is also a  
6  
7 340 need to understand how ecosystems depend on a particular organism or group of organisms for any  
8  
9 341 given process and function. This section describes the need to move from simply describing microbial  
10  
11 342 diversity to understanding what these organisms are doing, how they are doing it, and what biotic and  
12  
13 343 abiotic drivers are controlling their activity. Each question may derive a suite of different answers,  
14  
15 344 depending on the group of organisms, the habitat and the process.

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17 345

18  
19 346 36. What are the mechanisms driving microbial community structure and function, and are these  
20  
21 347 conserved across ecosystems?

22  
23 348 37. What is the relative importance of stochastic vs. determinative processes in microbial community  
24  
25 349 assembly?

26  
27 350 38. How conserved are microbial functions across different spatial and temporal scales?

28  
29 351 39. What is the relative importance of individual 'species' for the functioning of microbial communities?

30  
31 352 40. How much functional redundancy is there in microbial communities, and how does functional  
32  
33 353 redundancy affect measures of diversity and niche overlap?

34  
35 354 41. How often are functional traits of microbes successfully conferred through horizontal gene  
36  
37 355 transfer?

38  
39 356 42. What methods can we use to marry microbial diversity with function; how do we link  
40  
41 357 transcriptomics, proteomics and metabolomics?

42  
43 358 43. How do we move beyond correlation to develop predictive models that advance our understanding  
44  
45 359 of microbial community function and dynamics?"

46  
47 360 44. How useful are synthetic communities for testing theories about microbial community dynamics  
48  
49 361 and function?

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55 363 ***Evolutionary Processes***

56  
57 364 The role of microorganisms in determining evolutionary outcomes of hosts is being investigated in  
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3 365 increasing detail (McFall-Ngai *et al.*, 2013). Experimental evolution studies represent a powerful  
4 366 means of quantifying host-microbe and microbe-microbe coevolution, and have highlighted the  
5  
6 367 extraordinary capacity of microbes to act as key mediators of host fitness (e.g. King *et al.* 2016). Whilst  
7  
8 368 experimental coevolution studies provide a framework for linking dyadic interactions to community-  
9  
10 369 scale dynamics (Brockhurst & Koskella, 2013), evolutionary principles stemming from macro-ecology  
11  
12 370 are being applied to microbial communities of humans (Robinson *et al.*, 2010). However, fundamental  
13  
14 371 biological questions that are well-studied in macrobiology remain controversial for microbial ecology,  
15  
16 372 for example the species concept remains a source of debate (Freudenstein *et al.* 2016). The  
17  
18 373 operational taxonomic unit (OTU) has become the standard unit for identifying bacteria at the highest  
19  
20 374 taxonomic resolution possible, yet it is hard to clearly define where taxonomic boundaries lie between  
21  
22 375 two bacteria, and what an OTU really represents in biological terms. This is especially problematic in  
23  
24 376 the context of horizontal gene transfer, which is commonly observed in bacteria and has turned our  
25  
26 377 understanding of evolutionary processes upside down. This section relates to how general ecological  
27  
28 378 principles influence microbial evolution and *vice versa*, what this means for global biodiversity, and  
29  
30 379 whether evolutionary principles can be utilised for anthropogenic gain.

31 380

32  
33 381 45. How can a bacterial 'species' be defined?

34  
35 382 46. To what extent is faunal and floral biodiversity influenced by microbial communities?

36  
37 383 47. To what extent do microbial communities have an equivalent to keystone 'species'?

38  
39 384 48. Does the structure of microbial communities conform to the same ecological rules/principles as in  
40  
41 385 other types of communities?

42  
43 386 49. How do fundamental shifts in environmental conditions impact the trajectory of microbial  
44  
45 387 evolution?

46  
47 388 50. What are the relative selective forces favouring microbial genome expansion or reduction?

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49 389 **Society and Policy**

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51  
52 390 We need to find ways to apply fundamental biological research to the benefit of society and policy. For  
53  
54 391 example, collaboration with social scientists is crucial when investigating public understanding of  
55  
56 392 microbial ecology, as well as using citizen science approaches to tackle microbial ecology research  
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3 393 questions. Many questions relating to this area were discussed at the workshop, and here we present  
4 394 four additional questions that were developed at the meeting that relate to societal and policy-based  
5  
6 395 aspects of microbial ecology.  
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9 396

10  
11 397 • How can we best address supply and demand of information about microbial ecology between  
12  
13 398 researchers, clinicians, policy makers and practitioners?

14  
15 399 • How can we best use social and traditional mass media for early identification of emerging  
16  
17 400 threats to animal and plant health?

18  
19 401 • How can we develop an open access data repository or integrate existing databases to create  
20  
21 402 a centralised and standardised method for data and methods sharing in microbial ecology?

22  
23 403 • How can we replace fear-based regulation with risk-based regulation, specifically with regard  
24  
25 404 to the use of microbes in bioremediation and bioaugmentation?  
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## 30 406 **Discussion**

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33 407 Here we present 50 important research questions across a number of themes relating to the field of  
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35 408 microbial ecology. Although there are many other research issues worthy of investigation, it is  
36  
37 409 intended that these questions will be used to inform and direct future research programmes and  
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39 410 agendas, particularly in areas where microbial ecology has not previously been considered or applied.  
40  
41 411 In many cases, these questions are deliberately broad to allow researchers to adapt them to their own  
42  
43 412 areas of interest, for example across different systems, or to varying spatial scales. Across many  
44  
45 413 questions there was strong recognition of the vast metabolic capabilities of microorganisms and  
46  
47 414 microbial communities, and the need to harness this power to improve human and animal health and  
48  
49 415 wellbeing. Some themes addressed various existing mechanisms for exploiting microbial processes,  
50  
51 416 namely bioremediation, soil improvement, water treatment and probiotic suppression of pathogen  
52  
53 417 resistance. As these are already active areas of research, the questions posed here are structured to  
54  
55 418 provide a framework by which these efforts can be directed in the future.

56 419 A predominant theme that emerged was the need to integrate knowledge between different  
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58 420 research areas, for example the application of information from human microbiome studies to the  
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3 421 study of other non-model host organisms, and the potential to apply macro-ecological frameworks to  
4 422 micro-ecological concepts. Many fundamental biological questions that are well-studied in classical  
5 423 ecology remain controversial for microbial ecology, and the species concept (Freudenstein *et al.*  
6 424 2016), taxonomy, and how the OTU should be defined for microorganisms, generated multiple  
7 425 questions (e.g. see '*Evolutionary Processes*' theme). Classical community ecology concepts should  
8 426 not be overlooked when considering microbial dynamics (Rynkiewicz *et al.*, 2015) and, conversely,  
9 427 microbial communities may prove useful models for general ecology due to their short generation  
10 428 times, reproducibility, and ease of use in the laboratory environment (Brockhurst & Koskella, 2013;  
11 429 Libberton *et al.*, 2015; King *et al.*, 2016). There have been a number of calls for the medical profession  
12 430 to look to ecological and evolutionary tools when seeking to understand epidemiology (Johnson *et al.*,  
13 431 2015), investigating novel antibacterial agents (Vale *et al.*, 2016), and considering multi-host, multi-  
14 432 agent disease systems (Buhnerkempe *et al.*, 2015).

15  
16 433 The '*Host-Microbiome Interactions*' theme considered the need to understand factors  
17 434 influencing microbiome composition, which in turn have consequences for a myriad of host traits,  
18 435 including disease susceptibility and host evolution (Chisholm *et al.*, 2006; Archie & Theis, 2011; Spor  
19 436 *et al.*, 2011; Cho & Blaser, 2012; McFall-Ngai *et al.*, 2013; McFall-Ngai, 2015; Zilber-Rosenberg &  
20 437 Rosenberg, 2008). As this theme considered microbiota from the perspective of the host, there was  
21 438 some overlap with the '*Health and Infectious Diseases*' and '*Evolutionary Processes*' themes.  
22 439 Probiotics were discussed as a viable and promising alternative to current strategies in a number of  
23 440 contexts in these themes, not only to improve individual health, but also to decrease disease  
24 441 susceptibility of humans and other animals, to enhance nutritional quality of food, and to mitigate the  
25 442 negative impacts of antibiotic use across humans, livestock, aquaculture and agriculture (Martín *et al.*,  
26 443 2013; Newaj-Fyzul *et al.*, 2014; Smith, 2014; Fox, 2015). Developing personalized probiotic-based  
27 444 therapies requires complementary diversity and functional-based studies in order to elucidate the  
28 445 specific roles of microbiota in health and disease, and thus how microbial communities can be  
29 446 manipulated.

30  
31 447 Questions considered in both the '*Functional Diversity*' theme and the '*Environmental*  
32 448 *Processes*' theme raised a common need to understand changes in microbial community structure  
33 449 and function across spatial and temporal scales (Carmona *et al.*, 2016). Establishing appropriate  
34 450 spatial scales for studying microbial processes is an outstanding challenge: micro-organisms can

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3 451 orchestrate ecosystem functioning across whole biomes (Sheffer *et al.*, 2015), yet fungi exhibit low  
4 452 mobility on tree barks (Koufopanou *et al.* 2006, Robinson *et al.*, 2016), and an air void in soil can be  
5  
6 453 an insuperable barrier for a bacterium. Similarly, drawing meaningful conclusions about microbial  
7  
8 454 processes requires understanding of their temporal variability; for example, diurnal influences  
9  
10 455 (Shurpali *et al.*, 2016), or lags behind changes in ecosystem drivers (Allison and Martiny, 2008).

11  
12 456 A subject common to a number of themes was the role of individual species *versus* consortia  
13  
14 457 in community functioning. The question of defining bacterial species is a contentious topic, and the  
15  
16 458 issue remains whether some microbial taxa act as keystones in ecosystem functions. Many microbial  
17  
18 459 surveys carry the implicit assumption that the most abundant taxa are also the most important, yet  
19  
20 460 rare species can be hugely significant if they are highly active and/or monopolise a particular process  
21  
22 461 (Lynch and Neufeld, 2015). The collective metabolic capabilities of micro-organisms have great  
23  
24 462 potential for *in situ* applications such as bioremediation, particularly when used in multi-species  
25  
26 463 consortia (Mikesková *et al.*, 2012). Successful bioremediation and environmental management  
27  
28 464 requires the introduction of new assemblages into an established community, or stimulation of key  
29  
30 465 members of the community *in situ* (Rillig *et al.*, 2015). In turn, predicting the successful establishment  
31  
32 466 of deliberately introduced organisms depends on an understanding of the principles underlying  
33  
34 467 microbial community formation and structure. Despite these challenges, functional diversity modelling  
35  
36 468 has successfully been applied to the ecological restoration of some plant communities (Laughlin,  
37  
38 469 2014). Closely linked to this is the issue of functional redundancy, and to what extent it is possible to  
39  
40 470 lose species without affecting ecosystem functions. Already there is evidence that microbial  
41  
42 471 communities may be less functionally redundant than macro-organism communities (Delgado-  
43  
44 472 Baquerizo *et al.*, 2016). This issue ties into fundamental ecological concepts, such as niche theory  
45  
46 473 (Carmona *et al.*, 2016); if multiple organisms are carrying out the same process, apparently  
47  
48 474 interchangeably, how do they avoid competitively excluding one another? The concept of keystone  
49  
50 475 species has been shown to be applicable to microbes (Neufeld *et al.*, 2008; Pester *et al.*, 2010; Ze *et*  
51  
52 476 *al.*, 2012; Yu *et al.*, 2016), yet further work is needed to characterise the extent to which keystone  
53  
54 477 functions occur in different environments and whether these can be consistently identified (Anderson,  
55  
56 478 2003; Pester *et al.*, 2010).

57  
58 479 The need for open access databases and repositories, both in the context of data sharing as  
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60 480 well as for methods and protocols, was reflected in the questions shortlisted for the '*Society and*



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3 481 *Policy*' theme. Discussions included the benefits of forming collaborative and open research  
4 482 communities, and the need to ensure the legacy of academic research through improving regulation  
5 483 and policy and engagement with the public. Fear-based regulation of research, grounded in alarmist or  
6 484 populist campaigns, as opposed to risk-based regulation built upon evidence, was identified as a  
7 485 possible obstacle to progress, which could be addressed through greater interaction between  
8 486 microbial ecologists and the public at both governmental and grass roots levels. Large scale  
9 487 assessments of ecosystem services and degradation acknowledge the paucity of data on microbial  
10 488 impacts, presumably because there are no convincing large-scale messages that can be derived at  
11 489 this stage (Norris *et al.*, 2011). Microbial diversity is therefore rarely considered when estimates of  
12 490 biodiversity are required for policy or management decisions. That said, the increasing recognition of  
13 491 the fundamental impact of the microbial world on the functioning of larger-scale processes has made  
14 492 the deliberate manipulation of the microbial world a controversial subject, which was reflected in the  
15 493 number of draft questions submitted related to bioremediation and bioaugmentation (see  
16 494 Supplementary Information). Collaboration with social scientists was identified as crucial in gauging  
17 495 the public understanding of microbial ecology, and citizen science approaches were considered as  
18 496 tools to tackle key microbial ecology research questions.

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33 497         The 50 questions identified here cover a broad range of topics, but some over-arching themes  
34 498 recur across multiple questions, including a recognition that microbes play an important role in a  
35 499 variety of different processes and systems, which may be harnessed to solve real-world problems.  
36 500 There were some similarities between the questions identified here and those identified by previous  
37 501 workshops of a similar nature. For example, questions relating to soil health and biodiversity (Dicks *et*  
38 502 *al.* 2013), a requirement for developing a theoretical understanding of micro- and macro- ecological  
39 503 concepts (Prosser *et al.* 2007, Sutherland *et al.* 2013a) and disease dynamics (Prosser *et al.* 2007,  
40 504 Sutherland *et al.* 2013a) have a degree of commonality with this list. This indicates that the ecological  
41 505 theory underpinning many research questions transcends scientific disciplines, and that there is still  
42 506 much work to be done at both theoretical and applied levels. Within these 50 questions, we have tried  
43 507 to provide a focus for researchers addressing scientific questions from a microbial perspective,  
44 508 regardless of their background. It is expected that these questions will facilitate interesting discussion  
45 509 and new, exciting, interdisciplinary research. The list is by no means exhaustive, and we recognise  
46 510 that the questions presented here are relatively community-centric, primarily due to the recent

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3 511 expansion in methodological approaches that have improved our understanding of microbial  
4 512 community diversity and function. That said, other areas of microbial ecology should not be ignored or  
5 513 forgotten. Given the rapidly evolving field of microbial ecology, it is expected that future workshops  
6 514 with a wide draw will be held to ensure that the identification of research priorities and areas of interest  
7 515 is a continuing process.  
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39 529

### 40 41 42 530 **References**

43  
44 531 Allison, S, Martiny, J Resistance, resilience, and redundancy in microbial communities. *PNAS*,  
45 532 2008;**105 S1**, 11512-11519.  
46  
47 533 Anderson, TH Microbial eco-physiological indicators to asses soil quality. *Agric Ecosyst Environ*,  
48 534 2003;**98**, 285–293  
49  
50 535 Archie, EA, Theis, KR Animal behaviour meets microbial ecology. *Anim Behav*, 2011;**82**, 425-436  
51  
52 536 Baothman, OA, Zamzami, MA, Taher, I *et al.* The role of Gut Microbiota in the development of obesity  
53 537 and Diabetes. *Lipids Health Dis*, 2016;**15**, 108.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 538 Bissett, A, Brown, MV, Siciliano, SD *et al.* Microbial community responses to anthropogenically  
4 539 induced environmental change: towards a systems approach. *Ecol Lett*, 2013;**16**, 128-139.  
5  
6  
7 540 Boers, SA, Jansen, R, Hays, JP Suddenly everyone is a microbiota specialist. *Clin Microbiol Infect*,  
8 541 2016;**22**, 581-2.  
9  
10  
11 542 Bokulich, NA, Subramanian, S, Faith, JJ, *et al.* Quality-filtering vastly improves diversity estimates  
12 543 from Illumina amplicon sequencing. *Nat Methods*, 2013,**10**, 57-59  
13  
14  
15 544 Bragazza, L, Parisod, J, Buttler, A, *et al.* Biogeochemical plat-soil microbe feedback in response to  
16 545 climate warmings in peatlands. *Nat. Clim. Chang*, 2013;**3**, 273-277.  
17  
18  
19 546 Brockhurst MA, Koskella B Experimental coevolution of species interactions. *Trends Ecol Evol*,  
20 547 2013;**28**, 367–375  
21  
22  
23 548 Brown, S, Veach, A, Rigdon-Huss, A, *et al.* Scraping the bottom of the barrel: are rare high throughput  
24 549 sequences artifacts? *Fungal Ecol*, 2015;**13**, 221-225  
25  
26  
27 550 Buhnerkempe, MG, Roberts, MG, Dobson, AP, *et al.* Eight challenges in modelling disease ecology in  
28 551 multi-host, multi-agent systems. *Epidemics*, 2015;**10**, 26-30  
29  
30  
31 552 Buonaurio, R, Moretti, C, Passos da Silva, D, *et al.* The olive knot disease as a model to study the role  
32 553 of interspecies bacterial communities in plant disease. *Front. Plant Sci*, 2015;**6**: 434.  
33  
34  
35 554 Bustin, S, Benes, V, Garson, J, *et al.* The MIQE guidelines: minimum information for publication of  
36 555 quantitative real-time PCR experiments. *Clin. Chem*, 2009;**55**, 611-22  
37  
38  
39 556 Callahan, B, Proctor, D, Relam, D. *et al.* Reproducible research workflow in R for the analysis of  
40 557 personalised human microbiome data. *Pacific Symposium on Biocomputing*, 2016;**21**, 183-194.  
41  
42  
43 558 Carmona, C, de Bello, F, Mason, N, *et al.* Traits Without Borders: Integrating Functional Diversity  
44 559 Across Scales. *TREE*, 2016;**31**, 382-394  
45  
46  
47 560 Chaparro, JM., Sheflin, AM, Manter, DK, *et al.* Manipulating the soil microbiome to increase soil health  
48 561 and plant fertility. *Biol Ferti Soils*, 2012;**48**, 489-499  
49  
50  
51 562 Chibuike, GU, Obiora, SC Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods.  
52 563 *App Env Soil Sci*, 2014; Article ID 752708 1-12  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 564 Chin, JP, McGrath, JW, Quinn, JP. Microbial transformations in phosphate biosynthesis and  
4  
5 565 catabolism, and their importance in nutrient cycling. *Current Opinion in Chemical Biology*, 2016;**31**, 50-  
6  
7 566 57  
8  
9 567 Chisholm, ST, Coaker, G, Day, B, *et al.* Host-microbe interactions: Shaping the evolution of plant  
10  
11 568 immune response. *Cell* 2006;**126**, 803-814  
12  
13 569 Cho, I, Blaser, MJ. The human microbiome: at the interface of health and disease. *National Review of*  
14  
15 570 *Genetics*, 2013;**13**, 260-270  
16  
17 571 Coker, JA Extremophiles and biology: current uses and prospects. *F1000 Research*, 2016;**5**, F1000  
18  
19 572 FacultyRev-396  
20  
21 573 Creamer, CA, de Menezes, AB, Krull, ES, *et al.* Microbial community structure mediates response of  
22  
23 574 soil C decomposition to litter addition and warming. *Soil Biol Biochem*, 2015;**80**, 175-188  
24  
25 575 Daskin, JH, Alford, RA Context-dependent symbioses and their potential roles in wildlife diseases.  
26  
27 576 *Proc Bio Sci.* 2012;**279**, 1457-65  
28  
29 577 Delgado-Baquerizo, M, Giaramida, L, Reich, P, *et al.* Lack of functional redundancy in the relationship  
30  
31 578 between microbial diversity and ecosystem functioning. *J. Ecol*, 2016;**104**, 936–946  
32  
33 579 Dicks, LV, Abrahams, A, Atkinson, J, *et al.* Identifying key knowledge needs for evidence-based  
34  
35 580 conservation of wild insect pollinators: a collaborative cross-sectoral exercise. *Insect Conservation*  
36  
37 581 *and Diversity*, 2012;**6**, 435-446  
38  
39 582 Dicks, LV, Bardgett, RD, Bell, J, *et al.* What do we need to know to enhance the environmental  
40  
41 583 sustainability of agriculture? A prioritisation of knowledge needs for the UK food system. *Sustainability*,  
42  
43 584 2013;**5**, 3095–3115  
44  
45 585 Dumont, MG, Murrell, JC. Community-level analysis: key genes of aerobic methane oxidation.  
46  
47 586 *Methods Enzymol*, 2015;**397**, 413-427.  
48  
49 587 El Khawand, M, Crombie, AT, Johnston, A, *et al.* Isolation of isoprene degrading bacteria from soils,  
50  
51 588 development of isoA gene probes and identification of the active isoprene degrading soil community  
52  
53 589 using DNA-stable isotope probing. *Environ Microbiol*, 2016;**18**, 2743-53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 590 Ellouze, W, Esmaeili-Taheri, A, Bainard, LD, *et al.* Soil Fungal Resources in Annual Cropping Systems  
4 and Their Potential for Management. *BioMed Research International*, 2014;531824  
5  
6  
7 592 Freudenstein, JV, Broe, MB, Folk, *et al.* Biodiversity and the species concept-Lineages are not  
8  
9 593 enough. *Systematic Biology*, 2016;10.1093  
10  
11 594 Fox, JL. Agricultural probiotics enter spotlight. *Nature Biotechnology*, 2015;33, 122  
12  
13 595 Franklin AM, Aga, DS, Cytryn, E, *et al.* Antibiotics in agroecosystems: Introduction to the special  
14  
15 596 section. *J. Env Qual*, 2016;45, 377  
16  
17 597 Franzosa, E, Hsu, T, Sirota-Madi, A, *et al.* Sequencing and beyond: integrating molecular 'omics' for  
18  
19 598 microbial community profiling. *Nat. Rev. Microbiol*, 2015;13, 360-372  
20  
21 599 Fredriksson, N, Hermansson, M, Wilén, B The choice of PCR primers has great impact on  
22  
23 600 assessments of bacterial community diversity and dynamics in a wastewater treatment plant. *PLoS*  
24  
25 601 *One*, 2013;8, e76431  
26  
27  
28 602 Haritash, AK., Kaushik, CP Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A  
29  
30 603 review. *J Hazard Mater*, 2009;169, 1-15  
31  
32 604 Hartmann, M, Frey, B, Mayer, J, *et al.* Distinct soil microbial diversity under long-term organic and  
33  
34 605 conventional farming. *ISME J*, 2015;9, 1177-1194  
35  
36 606 Helgason, T, Daniell, TJ, Husband, R, *et al.* Ploughing up the wood-wide web? *Nature*, 1998;394, 431-  
37  
38 607 431  
39  
40 608 Hiraoka, S, Yang, CC, Iwasaki, W Metagenomics and bioinformatics in microbial ecology: current  
41  
42 609 status and beyond. *Microbes and Environments*, 2016;31, 204-212  
43  
44  
45 610 Hu, HW, Chen, D, He, JZ Microbial regulation of terrestrial nitrous oxide formation: understanding the  
46  
47 611 biological pathways for prediction of emission rates. *FEMS Microbiol Rev*, 2015;39, 729-749  
48  
49 612 Jacobsen, CS, Hjelmsø, MH. Agricultural soils, pesticides and microbial diversity. *Current opinion in*  
50  
51 613 *biotechnology*, 2014;27, 15-20  
52  
53 614 Johnson, PT, de Roode, JC, Fenton, A (2015). Why infectious disease research needs community  
54  
55 615 ecology. *Science*, 349, 1259504  
56  
57  
58  
59  
60

- 1  
2  
3 616 James, R. H., Bousquet, P., Bussmann, I., Haeckel, M., Kipfer, R., Leifer, I., Niemann, H., Ostrovsky,  
4  
5 617 I., Piskozub, J., Rehder, G., Treude, T., Vielstädte, L. and Greinert, J. Effects of climate change on  
6  
7 618 methane emissions from seafloor sediments in the Arctic Ocean: A review. *Limnol. Oceanogr.*, 2016;  
8  
9 619 **61**: S283–S299. doi:10.1002/lno.10307
- 10  
11 620 Jones, AC, Mead, A, Kaiser, MJ, *et al.* Prioritization of knowledge needs for sustainable aquaculture: a  
12  
13 621 national and global perspective. *Fish and Fisheries*, 2014;**16**, 668–683.
- 14  
15 622 Koch, H, Schmid-Hempel, P Socially transmitted gut microbiota protect bumble bees against an  
16  
17 623 intestinal parasite. *Proc Natl Acad Sci USA*, 2011;**108**, 19288–19292
- 18  
19 624 Koufopanou, V, Hughes, J, Bell, G, *et al.* The spatial scale of genetic variation in a model organism:  
20  
21 625 the wild yeast *Saccharomyces paradoxus*. *Phil Trans B* 2006;**29**, 1941-1946
- 22  
23 626 King, KC, Brockhurst, MA, Vasieva, O, *et al.* Rapid evolution of microbe-mediated protection against  
24  
25 627 pathogens in a worm host. *ISME J*, 2016;10.1038/ismej.2015.259
- 26  
27 628 Krishnasamy V, Otte J, Silbergeld E Antimicrobial use in Chinese swine and broiler poultry production.  
28  
29 629 *Antimicrob Resist Infect Control*, 2015;**4**, 17.
- 30  
31 630 Levy, SB Antibiotic resistance: an ecological imbalance. *Antibiotic resistance: Origins, Evolution,*  
32  
33 631 *Selection and Spread*. Wiley, Chichester: Ciba Foundation Symposium, 1997;**207**, 1-17
- 34  
35 632 Ley, RE, Hamady, M, Lozupone, C, *et al.* Evolution of mammals and their gut microbes. *Science*,  
36  
37 633 2008;**320**, 1647–1651
- 38  
39 634 Libberton, B, Horsburgh, MJ, Brockhurst, MA The effects of spatial structure, frequency dependence  
40  
41 635 and resistance evolution on the dynamics of toxin-mediated microbial invasions. *Evol. App*, 2015;**7**,  
42  
43 636 738-750
- 44  
45 637 Ling, L, Schneider, T, Peoples, T, *et al.* A new antibiotic kills pathogens without detectable resistance.  
46  
47 638 *Nature*, 2015;**517**, 455–459
- 48  
49 639 Lozupone, CA., Li, M, Campbell, TB, *et al.* Alterations in the gut microbiota associated with HIV-1  
50  
51 640 infection. *Cell host & microbe*, 2013;**14**, 329-339
- 52  
53 641 Lynch, M, Neufeld, J Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol*, 2015;**13**,  
54  
55 642 217–229
- 56  
57  
58  
59  
60



- 1  
2  
3 643 Martín, M, Miquel, S, Ulmer, J, *et al.* Role of commensal and probiotic bacteria in human health: a  
4 644 focus on inflammatory bowel disease. *Microbial Cell Factories*, 2013;**12**, 71  
5  
6  
7 645 McFall-Ngai, M, Hadeldb, MG, Boschc, TCG, *et al.* Animals in a bacterial world, a new imperative for  
8 646 the life sciences. *P Natl Acad Sci USA*, 2013;**110**, 3229-3236  
9  
10  
11 647 McFall-Ngai, MJ. Giving microbes their due--animal life in a microbially dominant world. *J Exp Biol*,  
12 648 2015;**218**, 1968-1973  
13  
14  
15 649 McMurdie, PJ, Holmes, S Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS*  
16 650 *Comput Biol*, 2014;**10**, e1003531  
17  
18  
19 651 Morris, CE, Bardin, M, Berge, O, *et al.* Microbial biodiversity: approaches to experimental design and  
20 652 hypothesis testing in primary scientific literature from 1975 to 1999. *Micr Mol Biol Rev*, 2002;**66**, 592-  
21 653 616  
22  
23  
24  
25 654 Mueller, K, Ash, C, Pennisi, E, *et al.* The gut microbiota. *Science*, 2012;**336**, 1245  
26  
27  
28 655 Newaj-Fyzul, A, Al-Harbi, H, Austin, B Review: Developments in the use of probiotics for disease  
29 656 control in aquaculture, *Aquaculture*, 2014;**431**, 1-11  
30  
31  
32 657 Neufeld, JD, Chen, Y, Dumont, MG, *et al.* Marine methylotrophs revealed by stable-isotope probing,  
33 658 multiple displacement amplification and metagenomics. *Environ Microbiol*, 2008;**10**, 1526–35  
34  
35  
36 659 Norris, K, Bailey, M, Baker, S, *et al.* Biodiversity in the context of ecosystem services. *In: The UK*  
37 660 *National Ecosystem Assessment Technical Report. UK National Ecosystem Assessment, UNEP-*  
38 661 *WCMC, Cambridge. 2011*  
39  
40  
41  
42 662 Oberhardt, M, Zarecki, R, Gronow, S, *et al.* Harnessing the landscape of microbial culture media to  
43 663 predict new organism–media pairings. *Nat. Comm*, 2015;**6**, 8493  
44  
45  
46 664 Oller, I, Malato, S, Sanchez-Perez, JA Combination of advanced oxidation processes and biological  
47 665 treatment for wastewater decontamination- a review. *Sci Total Environ*, 2011;**409**, 4141-4166.  
48  
49  
50  
51 666 Oulas, A, Pavludi, C, Polymenakou, P, *et al.* Metagenomics: tools and insights for analysing next-  
52 667 generation sequencing data derived from biodiversity studies. *Bioinform Biol Insights*, 2-15;**9**, 75-88  
53  
54  
55 668 Parsons, ECM, Favero, B, Aguirre, AA, *et al.* Seventy-one important questions for conservation of  
56 669 marine biodiversity. *Conserv Biol*, 2014;**28**, 1206–1214  
57  
58  
59  
60

- 1  
2  
3 670 Peay, K Back to the future: natural history and the way forward in modern fungal ecology. *Fungal Ecol*,  
4 671 2014;**2**, 4-9  
5  
6  
7 672 Pester, M, Bittner, N, Deevong, P, *et al.* A 'rare biosphere' microorganism contributes to sulfate  
8 673 reduction in a peatland. *ISME J*, 2010;**4**, 1591-602  
9  
10  
11 674 Prosser, JI, Bohannan, BJM, Curtis, TP, *et al.* The role of ecological theory in microbial ecology.  
12 675 *Nature*, 2007;**5**, 384-392  
13  
14  
15 676 Randle-Boggis, RJ, Helgason, T, Sapp, M, *et al.* Evaluating techniques for metagenome annotation  
16 677 using simulated sequence data. *FEMS Mic Ecol*, 2016;**92**,  
17  
18  
19 678 Rebollar, EA, Antwis, RE, Becker, MH, *et al.* Using "Omics" and Integrated Multi-Omics Approaches to  
20 679 Guide Probiotic Selection to Mitigate Chytridiomycosis and Other Emerging Infectious Diseases. *Front*  
21 680 *Microbiol*, 2016;**7**, 68  
22  
23  
24  
25 681 Redford, KH, Segre, JA, Salafsky, N, *et al.* Conservation and the microbiome. *Conserv Biol*, 2012;**26**,  
26 682 195-197  
27  
28  
29  
30 683 Ridaura, VK, Faith, JJ, Rey, FE, *et al.* Gut Microbiota from Twins Discordant for Obesity Modulate  
31 684 Metabolism in Mice. *Science*, 2013;**341**, 1241-1244  
32  
33  
34 685 Robinson, CJ, Bohannan, BJ, Young, VB From structure to function: the ecology of host-associated  
35 686 microbial communities. *Microbiol Mol Biol Rev*, 2010;**74**, 453-476  
36  
37  
38 687 Robinson, HA, Pinharanda, A, Bensasson, DB. Summer temperature can predict the distribution of  
39 688 wild yeast populations. *Ecol Evol*, 2016;**27**, 1236-1250  
40  
41  
42  
43 689 Round, JL, Mazmanian, SK. The gut microbiota shapes intestinal immune responses during health  
44 690 and disease. *Nat Rev Immunol*, 2009;**9**, 313-323  
45  
46  
47 691 Rynkiewicz, EC, Pedersen, A.B., Fenton, A An ecosystem approach to understanding and managing  
48 692 within-host parasite community dynamics. *Trends in Parasitology*, 2015;**31**, 212-221  
49  
50  
51 693 Sato, Y, Civiello, M, Bell, S, *et al.* Integrated approach to understanding the onset and pathogenesis  
52 694 of black band disease in corals. *Environmental Microbiology*, 2016;**18**, 752-765  
53  
54  
55 695 Schneider, T, Keiblinger, K, Schmid, E, *et al.* Who is who in litter decomposition? Metaproteomics  
56 696 reveals major microbial players and their biogeochemical functions. *ISME J*, 2012;**6**, 1749-1762  
57  
58  
59  
60

- 1  
2  
3 697 Schuur, EAG, McGuire, AD, Schädel, C *et al.* Climate change and the permafrost carbon feedback.  
4  
5 698 *Nature*, 2015;**520**, 171-179  
6  
7 699 Serino, M, Nicholas, M, Trabelsi, MS, *et al.* Young microbes for adult obesity. *Pediatric Obesity*,  
8  
9 700 2016;10.1111.Sheffer, E, Batterman, S, Levin, S, *et al.* Biome-scale nitrogen fixation strategies  
10  
11 701 selected by climatic constraints on nitrogen cycle. *Nat Plants*, 2015;**1**, 15182  
12  
13 702 Shurpali, N, Rannik, U, Jokinen, S, *et al.* Neglecting diurnal variations leads to uncertainties in  
14  
15 703 terrestrial nitrous oxide emissions. *Sci Rep*, 2016;**6**, 25739  
16  
17 704 Singh, BK., Bardgett, RD., Smith, P, *et al.* Microorganisms and climate change: terrestrial feedbacks  
18  
19 705 and mitigation options. *Nat Rev Microbiol*, 2010;**8**, 779-790  
20  
21 706 Smith, JMA Review of Avian Probiotics, *Journal of Avian Med Surg*, 2014;**28**, 87-94  
22  
23 707 Smith, DP, Peay, KG Sequence depth, not PCR replication, improves ecological inference from next  
24  
25 708 generation sequencing. *PLoS One*, 2014;**9**, e09234  
26  
27 709 Smits, HH, Hiemstra, PS, Prazeres da Costa, C, *et al.* Microbes and asthma: Opportunities for  
28  
29 710 intervention. *J Allergy Clin Immunol*, 2016;**137**(3), 690-697  
30  
31 711 Spor, A, Koren, O, Ley, R Unravelling the effects of the environment and host genotype on the gut  
32  
33 712 microbiome. *Nat Rev Microbiol*, 2011;**9**, 279-290  
34  
35 713 Sutherland, WJ, Adams, WM, Aronson, RB, *et al.* One hundred questions of importance to the  
36  
37 714 conservation of global biological diversity. *Conserv Biol*, 2009;**23**, 557-567  
38  
39 715 Sutherland, WJ., Fleishman, E, Mascia, MB, *et al.* Methods for collaboratively identifying research  
40  
41 716 priorities and emerging issues in science and policy. *MEE*, 2011;**2**, 238-247  
42  
43 717 Sutherland, WJ, Armstrong-Brown, S, Armsworth, PR, *et al.* Identification of 100 fundamental  
44  
45 718 ecological questions. *J Ecol*, 2013a;**101**, 58-67  
46  
47 719 Sutherland, WJ, Goulden, C, Bell, K, *et al.* 100 Questions: Identifying research priorities for poverty  
48  
49 720 prevention and reduction. *J Poverty Soc Justice*, 2013b;**21**, 189-205  
50  
51 721 Thomas, V, McDonnell, G, Denyer, SP, *et al.* Free-living amoebae and their intracellular pathogenic  
52  
53 722 microorganisms: risks for water quality. *FEMS Microbiol Rev*, 2010;**34**, 231-259  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 723 Thomas, T, Gilbert, J, Meyer, F Metagenomics – a guide from sampling to data analysis. *Microb*  
4 724 *Inform Exp*, 2012;**2**, 3  
5  
6  
7 725 Ubeda, C, Bucci, V, Caballero, S, *et al.* Intestinal microbiota containing *Barnesiella* species cures  
8 726 vancomycin-resistant *Enterococcus faecium* colonization. *Infect Immun*, 2013;**81**, 965-973.  
9  
10  
11 727 Vale, PF., McNally, L, Doeschl-Wilson, A, *et al.* Beyond Killing: Can we find new ways to manage  
12 728 infection? *Evol Med Public Health*, 2016; doi: 10.1093/emph/eow012  
13  
14  
15 729 Vayssier-Taussat M, Albina E, Citti C, *et al.* Shifting the paradigm from pathogens to pathobiome: new  
16 730 concepts in the light of meta-omics. *Front Cell Infect Microbiol*, 2014;**4**, 29  
17  
18  
19 731 Walter, J, Ley, R The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev*  
20 732 *Microbiol*, 2011;**65**, 411-429  
21  
22  
23 733 Weiss, B, Aksoy, S. Microbiome influences on insect host vector competence. *Trends in Parasitology*,  
24 734 2011;**27**, 514–522  
25  
26  
27  
28 735 Weiss, S, Van Treuren, W, Lozupone, C, *et al.* Correlation detection strategies in microbial data sets  
29 736 vary widely in sensitivity and precision. *ISME J*, 2016a;**10**, 1669–1681  
30  
31  
32 737 Weiss, S, Xu, Z, Amir, A, *et al.* Effects of library size variance, sparsity, and compositionality on the  
33 738 analysis of microbiome data. *Peer J*, 2016b;**3**, e1408  
34  
35  
36 739 WHO. Tackling Antibiotic Resistance from a Food Safety Perspective in Europe. Copenhagen,  
37 740 Denmark: WHO Regional Office for Europe 2011. Available:  
38 741 [http://www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety-  
40 741 perspective-in-europe](http://www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety-<br/>39 742 perspective-in-europe) [accessed 19 July 2016].  
41  
42  
43  
44 743 Widder, S, Widder, S, Allen, RJ *et al.* Challenges in microbial ecology: building predictive  
45 744 understanding of community function and dynamics. *ISME J*, 2016;DOI: 10.1038/ismej.2016.45  
46  
47  
48 745 Wieder, WR, Bonan, GB, Allison, SD Global soil carbon projections are improved by modelling  
49 746 microbial processes. *Nat Clim Change*, 2013;**3**, 909-912  
50  
51  
52 747 Willing, BP, Russell, SL, Finlay, BB Shifting the balance: antibiotic effects on host–microbiota  
53 748 mutualism. *Nat Rev Microbio*, 2011;**9**, 233-243  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 749 Yu, Z, Krause, SMB, Beck, DAC, *et al.* Synthetic Ecology Perspective: How Well Does Behavior of  
4 750 Model Organisms in the Laboratory Predict Microbial Activities in Natural Habitats? *Front Microbiol*,  
5 751 2016;**7**, 1–7  
6  
7  
8 752 Ze, X, Duncan, SH, Louis, P, *et al.* *Ruminococcus bromii* is a keystone species for the degradation of  
9 753 resistant starch in the human colon. *ISME J*, 2012;**6**, 1535–1543  
10  
11 754 Zilber-Rosenberg, I, Rosenberg, E Role of microorganisms in the evolution of animals and plants: the  
12 755 hologenome theory of evolution. *FEMS Micro Rev*, 2008;**32**, 723-735  
13  
14  
15 756 Zuber, SM, Villamil, MB Meta-analysis approach to assess effect of tillage on microbial biomass and  
16 757 enzyme activities. *Soil Biology and Biochemistry*, 2016;**97**, 176-187  
17  
18  
19  
20  
21  
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7 **1 50 important research questions in microbial ecology**

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31 38 Keywords:  
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33 39 environmental processes, evolutionary processes, functional diversity, host-microbiome interactions,  
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35 40 ~~methods~~, priority setting, research agenda  
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41 **Abstract**

42 Microbial ecology provides insights into the ecological and evolutionary dynamics of microbial  
43 communities underpinning every ecosystem on Earth. Microbial communities can now be investigated  
44 in unprecedented detail, although there is still a wealth of open questions to be tackled. Here we  
45 identify 50 research questions of fundamental importance to the science or application of microbial  
46 ecology, with the intention of summarising the field and bringing focus to new research avenues.  
47 Questions are categorised into [eight seven](#) themes: Host-Microbiome Interactions; Health and  
48 Infectious Diseases; ~~Food Security and Human Health~~ [and Food Security](#); Microbial Ecology in a  
49 Changing World; Environmental Processes; Functional Diversity; ~~and~~ Evolutionary Processes; ~~and~~  
50 ~~Methods in Microbial Ecology~~. Many questions recognise that microbes provide an extraordinary array  
51 of functional diversity that can be harnessed to solve real-world problems. Our limited knowledge of  
52 spatial and temporal variation in microbial diversity and function is also reflected, as is the need to  
53 integrate micro- and macro-ecological concepts, and knowledge derived from studies with humans  
54 and diverse other organisms. Certain methods remain inadequate and currently limit progress in the  
55 field. Although not exhaustive, the questions presented are intended to stimulate discussion and  
56 provide focus for researchers, funders, and policy makers, informing the future research agenda in  
57 microbial ecology.

58

## 59 Introduction

60 In recent years, there has been an explosion in microbial ecological research, which is reflected in  
61 broad-scale research projects such as the Human Microbiome Project and the Earth Microbiome  
62 Project, as well as in the peer-reviewed literature (e.g. Boers et al., 2016). Recent rapid technological  
63 advances, including next-generation sequencing, (meta)genomics, metabolomics,  
64 (meta)transcriptomics and (meta)proteomics, have vastly increased our ability to study microbial  
65 community complexity and function (Morris *et al.*, 2002; Hiraoka *et al.*, 2016). These provide  
66 unprecedented opportunities to assess genomic potential, gene regulation, expression and function *in*  
67 *situ* (Schneider *et al.*, 2012, Franzosa *et al.*, 2015), especially when combined with detailed knowledge  
68 of natural history and environmental parameters (Peay, 2014). Such techniques have been applied to  
69 a vast range of fields within the scope of 'microbial ecology' in order to better understand how  
70 microorganisms interact with and affect their environment, each other, and other organisms.

71 With an overwhelming and ever-growing number of potential and critical research avenues in  
72 microbial ecology, it is timely to identify major questions and research priorities that would progress  
73 the field. Here we present the results of a workshop hosted by the British Ecological Society's  
74 Microbial Ecology Special Interest Group in June 2016, which used a discussion and voting-based  
75 system to identify 50 research questions of importance to the field of microbial ecology. Similar  
76 exercises identifying important research questions have been conducted in conservation (Sutherland  
77 *et al.*, 2009, Dicks et al. 2012), pure ecology (Sutherland *et al.*, 2013a), marine biodiversity (Parsons  
78 *et al.*, 2014), sustainability (Dicks *et al.*, 2013; Jones et al. 2014), and non-ecological subjects  
79 including UK poverty (Sutherland *et al.*, 2013b). These papers have been widely accessed and are  
80 directly applicable to the development of policy, as highlighted by Jones *et al.* (2014).

81

82

## 83 Methods

### 84 *Participants*

85 The methods used here were based broadly on those presented in Sutherland *et al.* (2011). A one-day  
86 workshop was held by the British Ecological Society's Microbial Ecology Special Interest Group at the  
87 University of Salford (UK) in June 2016. Invitations to attend the meeting were distributed via the

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7 88 British Ecological Society's membership mailing list and through social media (Twitter and Facebook).

8 89 In total, 34 participants from 20 institutions attended and contributed to the development of the 50

9 90 questions listed below, with the majority listed as authors on this paper.

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14 92 *Questions*

15  
16 93 Prior to the workshop, attendees were asked to submit questions via an online form that they thought

17 94 most closely met the following brief:

18  
19 95 "We are aiming to identify 50 questions that, if answered, will make a considerable

20 96 difference to the use of microbial ecology by practitioners and policy makers, or to

21 97 the fundamentals of the field of microbial ecology. These should be questions that

22 98 are unanswered, could be answered, and could be tackled by a research

23 99 programme. This is expected to set the agenda for future research in the field of

24 100 microbial ecology."  
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31 102 A total of 244 questions were submitted by attendees (see Supplementary Information), and assigned

32 103 (by R.E. Antwis and S.M. Griffiths) to the following ~~eight~~ themes;

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34  
35 104 1) *Host-Microbiome Interactions*;

36  
37 105 2) *Health and Infectious Diseases*;

38  
39 106 3) ~~*Food Security and Human Health*~~ *and Food Security*;

40  
41 107 4) *Microbial Ecology in a Changing World*;

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43 108 5) *Environmental Processes*;

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45 109 6) *Functional Diversity*;

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47 110 7) *Evolutionary Processes*;

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49 111 ~~8) *Methods in Microbial Ecology*.~~

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53 113 An additional ~~ninth~~ ~~eighth~~ theme named '*Society and Policy*' was created to capture a number of

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7 114 questions that were generally applicable across the biological sciences, as well as a number of  
8 115 questions specific to the field of microbial ecology which could not necessarily be addressed through  
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10 116 laboratory based microbial ecology research, *per se*.

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14 118 *Question selection process*

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16 119 Prior to the workshop, participants were asked to identify the top ~20% of questions in each theme  
17 120 that most closely aligned with the brief (selection of 5-11 questions from a total of 26-57 questions per  
18 121 theme via online form; Supplementary Information). Participants were asked to consider all questions  
19 122 within a theme and to select questions based on the theme's context and the brief for the workshop.  
20 123 Some questions were included in more than one theme to encourage discussion and to increase the  
21 124 likelihood that pertinent questions remained in the selection process. Questions were then ranked  
22 125 according to the number of online votes they received, and this formed the material for the workshop.

23  
24 126 Three sets of parallel sessions were run at the workshop, with participants free to select which  
25 127 theme sessions they attended. Questions were discussed in order of lowest ranking to highest, with  
26 128 duplicates removed and questions reworded as necessary. For each theme, a final set of 'gold' (~15%  
27 129 of questions, total of 47 questions across all themes) and 'silver' questions (~10% of questions, total of  
28 130 29 questions) were identified. Where necessary, a show of hands was used to ensure the democratic  
29 131 process was upheld.

30 132 A final plenary session was held in which all gold and silver questions were discussed. For  
31 133 gold questions, duplicates among categories were removed and questions reworded to reflect the  
32 134 discussion in the room, resulting in 43 gold questions. A similar process was then completed for silver  
33 135 questions, and a show of hands used to vote for seven questions that could be elevated to gold status  
34 136 to form the final set of 50 questions ~~across the eight themes~~.

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39 138 *Limitations*

40 139 All but four participants were from British universities, although there were representatives from a  
41 140 range of nationalities and research areas. The manner in which this paper was developed (*i.e.* through  
42 141 a physical workshop and via the British Ecological Society) means that, without a substantial travel

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7 142 budget, a bias towards UK institutions was inevitable. However, many participants have worked on, or  
8 143 currently collaborate in, research projects on non-UK ecosystems and species, and therefore the  
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10 144 questions proposed are drawn from considerable knowledge and experience of the field  
11 145 internationally. Additionally, although most individuals were from academic institutions, many  
12  
13 146 individuals had previous or on-going collaborations with industrial partners and governmental/non-  
14  
15 147 governmental organisations.  
16  
17 148

## 18 149 **Results**

20 150 The following 50 questions are presented by theme, and are not ordered according to relevance or  
21  
22 151 importance. Due to the nature of the process, some questions may appear similar across themes, but  
23  
24 152 within the context of each theme can take on a different meaning. Some questions may relate to  
25  
26 153 research areas that are already somewhat active, and these serve to highlight the importance of and  
27  
28 154 encourage further work in these areas. Some of these questions apply across multiple biomes and  
29  
30 155 ecosystems, and can be considered in the context of multiple host organisms and across varying  
31  
32 156 temporal and spatial scales.  
33

### 34 158 **Host-Microbiome Interactions**

36 159 Host-microbiome interactions determine many host life history traits such as behaviour, reproduction,  
37  
38 160 physiological processes, and disease susceptibility (Archie and Theis, 2011; Willing *et al.*, 2011; Koch  
39  
40 161 & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.*, 2016). ~~The 'hologenome theory' suggests~~  
41 162 ~~that the microbiome be considered an integral part of the host system, with the evolution of an~~  
42  
43 163 ~~individual's own genetic material and that of the associated microbiota intrinsically linked (Zilber-~~  
44  
45 164 ~~Rosenberg & Rosenberg, 2008; Daskin & Alford, 2012).~~ Increasingly, we are discovering that host-  
46 165 microbiome interactions produce complex and dynamic communities that fluctuate in compositional  
47  
48 166 abundance ~~influenced by correlated with~~ factors as diverse as host genotype, developmental stage,  
49  
50 167 diet, and temporal changes, among others (e.g. Spor *et al.*, 2011). Even in otherwise well studied  
51  
52 168 organisms, very little is known about the consequences of microbiome variation for host processes,  
53  
54 169 particularly across different spatial and temporal scales. Considerations of host microbiomes are also  
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56 170 likely important for global issues, such as the efficacy of conservation efforts including species



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7 171 reintroduction programmes (reviewed in Redford *et al.*, 2012; McFall-Ngai, 2015). Additionally,  
8 172 interactions between native and non-native species are ~~affected by~~correlated with transmission of  
9  
10 173 microbiota, often determined by relatedness or diet type (Ley *et al.*, 2008), and the microbiome plays a  
11 174 key role in the control and competence of insect crop pests and vectors of disease (reviewed in Weiss  
12 175 & Aksoy, 2011). The following questions aim to address the shortfall in our understanding of the  
13 176 interactions between microbiomes and their human and non-human hosts.

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20 179 1.4. What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe  
21  
22 180 interactions?

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23  
24 181 2. What are the relative contributions of host-associated and environmental factors in determining host  
25  
26 182 microbial community composition?

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27  
28 183 3.2. How do microbial communities function to affect the phenotype of the host?

29  
30 184 4.3. Can compositional or evolutionary changes in microbiomes help hosts adapt to environmental  
31 185 change within the lifetime of the host?

32  
33 186 5.4. What is the role of the microbiota in host speciation processes?

34 187 6.5. How can the associated microbiota be effectively included in risk assessments of Invasive Non-  
35 188 Native Species?

36  
37 189 7.6. How does the microbiome of captive ~~and head-started~~ animals affect the success of reintroduction  
38 190 programmes?

39 191 8. How can a 'systems biology' approach improve our understanding of host-microbe interactions?

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41 193

#### 42 194 **Health and Infectious Diseases**

43 195 The last 50 years have seen the emergence of several hypervirulent wildlife pathogens in animals  
44 196 (e.g. Tasmanian devil face tumour disease, avian malaria, amphibian chytridiomycosis; reviewed in  
45 197 Tompkins *et al.*, 2015) and plants (e.g. sudden oak and larch death, ash dieback; Pautasso *et al.*,

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7 198 2015). Although the role of microorganisms as pathogens is well known, the importance of host-  
8 199 associated microbiomes in regulating disease susceptibility is becoming more apparent (Koch &  
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10 200 Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.*, 2016). A major outstanding research goal is  
11 201 to understand how within-host interactions among microbes and invading pathogens may shape  
12  
13 202 patterns of infection intensity and disease progression (see also *Evolutionary Processes*). Several  
14  
15 203 studies have sought to determine how manipulation of host microbiomes may ameliorate the spread  
16 204 and impact of such diseases (e.g. Rebollar *et al.*, 2016).

17  
18 205 While for many disease states the paradigm holds true that one microorganism causes one  
19  
20 206 disease, polymicrobial infections are becoming more apparent through metagenomic and  
21 207 metatranscriptomic sequencing of disease-associated microbial communities (Gilbert *et al.*, 2016).  
22  
23 208 Consequently, the “pathobiome” concept, where a disease state is influenced by complex  
24  
25 209 interactions between commensal and pathogenic microorganisms, presents new challenges for  
26 210 applying Koch’s postulates to diseases arising from polymicrobial interactions (Vayssier-Taussat *et al.*,  
27  
28 211 2014), such as black band disease (BBD) in corals (Sato *et al.*, 2016) and olive knot disease  
29 212 (Buonaurio *et al.*, 2015).

30  
31 213 In this ~~section-theme~~ we have identified research questions relating to the microbial ecology of  
32  
33 214 infectious diseases and host health. Although much can be learnt from the comparatively high number  
34  
35 215 of studies in the human and biomedical literature (e.g. using network approaches in epidemiology), the  
36 216 questions selected in this theme predominantly relate to non-human animals and plants, as humans  
37  
38 217 are covered later (*Food Security and Human Health and Food Security*).

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42 219 [948. How can we better track the source and dispersal of particular microorganisms in real time?](#)

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44 220  
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46 221  
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48 222 [107](#). Many microorganisms are unculturable, and many microbiome studies reveal that diseases are  
49  
50 223 polymicrobial; how can we re-evaluate Koch's postulates in this context?

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52 224 [118](#). Which factors trigger ‘covert’ infections to become ‘overt’, impacting host health?

53  
54 225 [129](#). At the population level, how is the burden and shedding intensity of intracellular microbes

226 affected by co-infection by extracellular parasites?

227 ~~134~~0. What is the ecological relevance of the internalization of bacterial pathogens by protozoa in  
228 terms of their survival and spread?

229 ~~14~~1. How can network theory best be used to predict and manage infectious disease outbreaks in  
230 animals and plants?

231 ~~15~~2. Can microbiomes of wildlife (plants and animals) be used or manipulated to enhance health  
232 and/or disease resistance?

233

234 Human Health and ~~Human Health and~~ Food Security ~~and Human Health~~

235 With the human population due to exceed eight billion by 2024, food security and human health are  
236 high on political and scientific agendas. ~~Though the remit for this section is relatively broad, the  
237 questions focus on two central themes: i) studying the human microbiome to improve the treatment of  
238 disease, including the development of personalized medicine and novel antibiotics; and ii)  
239 understanding how current antibiotic regimes and farming practices may negatively impact the  
240 diversity of the environmental microbiome and food production capacity.~~

241 The human microbiome has been the focus of intense research efforts in recent years, (e.g. Walter &  
242 Ley, 2011; Spor *et al.*, 2011; Mueller *et al.*, 2012), because gut symbionts shape the immune  
243 response (Round *et al.*, 2009), and diversity fluctuates through chronic conditions and infectious  
244 diseases including diabetes, obesity (Serino *et al.* 2016; Baothman *et al.*, 2016; Ridaura *et al.*, 2013),  
245 asthma (Smits *et al.* 2016), and HIV (Lozupone *et al.*, 2013). Improving our understanding of the core  
246 human microbiome and individual variation will underpin pharmomicrobiomics, enabling development  
247 of novel therapeutic treatments and, ultimately, personalised medicine (e.g. Ubeda *et al.*, 2013).

248 ~~There was a strong interest in maintaining and enhancing the microbial populations of crop  
249 ecosystems, especially in light of antibiotic resistance (Ellouze *et al.*, 2014). As antibiotic resistance  
250 increases along with our concern about potential impact on both human and animal health, there is an  
251 increasing drive to find new forms of antibiotics.~~

252 Antibiotic resistance resulting from selective pressures generated by the use and misuse of  
253 antibiotics is a global threat to public health (Levy, 1997; Tam *et al.*, 2012). The volume of antibiotics

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7 254 used in agriculture now exceeds the amount used in human medicine in many countries (WHO, 2011).  
8 255 Antibiotics are still widely used in livestock for prophylaxis and growth promotion, often at sub-  
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10 256 therapeutic concentrations, exacerbating resistance (Krishnasamy *et al.*, 2015). The impact of the  
11 257 leaching of antibiotics into the natural environment and subsequent impacts on natural microbial  
12  
13 258 communities remains poorly characterised (Franklin *et al.*, 2016). Current practices of growing high-  
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15 259 intensity monoculture crops have a negative impact on the microbial biodiversity of soils through a  
16 260 combination of tillage, subsequent erosion and chemical applications (Helgason *et al.*, 1998; Jacobsen  
17  
18 261 and Hjelmsø, 2014; Zuber and Villamil, 2016), which imposes selection pressures on pathogenic  
19 262 microbes, fungal symbiotic partners and plant growth promoting bacteria (Chapparo *et al.*, 2012;  
20  
21 263 Hartmann *et al.*, 2015). ~~Thus, there iwas a strong interest ineed to maintaining and enhanceing the~~  
22 264 ~~microbial populations of crop ecosystems, especially in light of antibiotic resistance (Ellouze *et al.*,~~  
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24 265 ~~2014). As antibiotic resistance increases, along with our concern about potential impact on both~~  
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26 266 ~~human and animal health, there is an increasing drive to find new forms of antibiotics.~~

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28 267  
29  
30 268 ~~Though the remit for this section is relatively broad, the questions focus on two central~~  
31 269 ~~themes: i) studying the human microbiome to improve the treatment of disease, including the~~  
32  
33 270 ~~development of personalized medicine and novel antibiotics; and ii) understanding how current~~  
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35 271 ~~antibiotic regimes and farming practices may negatively impact the diversity of the environmental~~  
36 272 ~~microbiome and food production capacity. This section examines ways in which we can harness~~  
37  
38 273 ~~microbial functions to improve overall human health through managing gut microbiota, and improve~~  
39 274 ~~the soil and plant microbiome, thus increasing yields and associated biodiversity.~~

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43 276 163. How can human microbiome studies improve personalised medicine?

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45 277 174. What ecological principles can be applied in the search for new antibiotics and alternatives?

46  
47 278 185. What are the main determinants of waterborne infection outbreaks, and what is the best strategy  
48  
49 279 to control these in water distribution systems?

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51 280 196. What are the consequences of antibiotic and pharmaceutical use in human medicine on microbial  
52  
53 281 communities in freshwater and soil environments?

282 [20. To what extent are microbial species distributions influenced by climate, and what are the](#)  
283 [consequences for food security and human health?](#)

284 [2147](#). How much microbial diversity in the soil has been lost through monoculture and what is the  
285 importance of this?

286 [2248](#). Intensive farming may involve high levels of agrochemicals and broad-spectrum antibiotic usage  
287 - what will be the long-term effects on microbial communities?

288 [2349](#). How best can we harness microbial communities to enhance food production?

289

### 290 ***Microbial Ecology in a Changing World***

291 [Global changes resulting from human activity impact almost every Earth-habitat on earth. It is](#)  
292 [imperative that we focus efforts on understanding the impacts of human activities such as climate](#)  
293 [change, urbanisation, agriculture, and industrial processes on microbial communities, ecosystem](#)  
294 [functioning equilibrium, and host health. Microbial populations have a tremendous capacity to adapt to](#)  
295 [changes in their abiotic environment, yet the functional implications of these transitions in microbial](#)  
296 [ecology are still poorly understood and characterised \(Bissett \*et al.\*, 2013\), and the role of microbes in](#)  
297 [mediating the response of larger organisms to change is equally understudied. Plant associated](#)  
298 [microbial communities can alter the performance of subsequent generations of plants, and can](#)  
299 [themselves be subject to the concomitant effects of abiotic change \(van der Putten \*et al.\*, 2013\). Such](#)  
300 [plant-soil feedbacks can reduce yield and alter the community composition and invasibility of whole](#)  
301 [areas of grassland \(van der Putten \*et al.\*, 2013\).](#) Global environmental changes (GECs) are complex  
302 and multifaceted. Human activities such as urbanisation, land-use change and introduction of invasive  
303 species have played a role in shifting global ecosystems via desertification, climate change and  
304 habitat degradation. Although such changes have been quantified in aquatic and terrestrial habitats  
305 (e.g. Haberl *et al.*, 2007; Halpern *et al.*, 2008), their effects on microbial communities and impacts on  
306 ecosystem function are often hindered by a lack of characterisation of communities, or limited  
307 understanding of microbial functional traits. Shifts in basic nutrients and gases such as CO<sub>2</sub>, along  
308 with temperature fluctuations and water availability, greatly influence the distribution and behaviour of  
309 species (Tylianakis *et al.*, 2008). GECs can alter host fitness or ecosystem functioning (Shay *et al.*,  
310 2015; Webster *et al.* 2016) and are likely to occur in combination. While there is a great deal of

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7 311 research into the effects of each of these on microbial communities (Schimel *et al.*, 2007; Shurin *et al.*,  
8 312 2012; Lloret *et al.*, 2014), literature considering the effect of multiple GECs is ~~more sparse~~sparser, and  
9  
10 313 these have complicated and often unpredictable consequences when combined (although see  
11 314 Hutchins *et al.*, 2009; Ryalls *et al.*, 2013). In this section, we consider how human activities directly  
12 315 and indirectly influence the microbial world. Where applicable, these questions can be considered  
13 316 across multiple biomes and ecosystems, with reference to resulting trophic cascades, in addition to  
14 317 the impacts on multiple biogeochemical processes. We also consider how microbes can be used as a  
15 318 tool for mitigation or bioremediation of human-induced environmental changes, and the ways in which  
16 319 microbes can be included in current evaluations of global change.  
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23 321 240. How can we integrate microbial communities into models of global change?

24 322 254. Will ocean acidification, temperature increases and rising sea levels lead to changes in microbial  
25 323 diversity or function, and what will the cascading effects of this be?

26 324 262. How do human activities, such as oil and gas drilling, influence the sub-surface microbiome(s)?

27 325 273. How will increasing urbanisation affect environmental and host-associated microbial  
28 326 communities?

29 327 28. How resilient are different microbial functional groups to ecosystem disturbance?

30 328 294. Can we manipulate microbial succession in species-poor soils to encourage repopulation by flora  
31 329 and fauna?  
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### 331 **Environmental Processes**

332 Microbes play a fundamental role in environmental processes and ecosystem services, including  
333 nutrient cycling and organic matter decomposition (Chin *et al.* 2016; Creamer *et al.*, 2015; Weider *et al.*,  
334 2013), bioremediation of contaminated habitats or waste systems (Haritash & Kaushik, 2009; Oller  
335 *et al.*, 2011), and influencing greenhouse gas emissions (Singh *et al.*, 2010; Bragazza *et al.*, 2013; Hu  
336 *et al.*, 2015). The ability to harness these processes has great potential for societal and environmental  
337 applications, particularly in extremophiles, which frequently reveal metabolic capabilities and  
338 evolutionary solutions not witnessed elsewhere in the microbial world (Coker *et al.* 2016). However, it  
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7 339 is rarely possible to directly link the presence of a specific microbial taxon to a particular ecological  
8 340 process. Other methodological challenges include establishing the relative importance of biotic and  
9 341 abiotic factors in microbial ecosystem function, and determining the appropriate spatial and temporal  
10 342 scale necessary to discriminate links between microbiota and their ecological functions (Bissett *et al.*,  
11 343 2013). Concurrently, a deeper understanding is required of human-induced impacts on the global  
12 344 microbiome through urbanisation, habitat degradation, climate change, and the introduction of invasive  
13 345 species, amongst others.  
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20 347 [3025](#). How do we successfully establish microbial communities used in bioremediation?

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22 348 [3126](#). How important is the rare microbiome in ecosystem function, and how does this change with  
23 349 stochastic events?

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25  
26 350 [3227](#). To what extent is microbial community diversity and function resilient to short- and long-term  
27 351 perturbations?

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29 352 [3328](#). What is the importance of spatial and temporal variation in microbial community structure and  
30 353 function to key environmental processes and geochemical cycles?

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32 354 [3449](#). How can we accurately measure microbial biomass in a reproducible manner?

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35 355  
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37 356 [3529](#). Which mechanisms do extremophiles use for survival and how can they be exploited?  
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### 39 40 41 358 **Functional Diversity**

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43 359 Ecologists are increasingly turning their attention to classifying species based on their activity  
44 360 (function) within an ecosystem, rather than their genotype (Crowther *et al.*, 2014). This is particularly  
45 361 relevant for microbial ecology, in which species are hard to define, horizontal gene transfer is rife, and  
46 362 taxonomy is often blurred. Understanding how membership within complex and dynamic microbial  
47 363 communities relates to the function of that community is one of the key challenges facing microbial  
48 364 ecology (Widder *et al.*, 2016). This is true across a vast range of spatial scales, from microbial dyads  
49 365 to the gut of a *Drosophila* fly, to ancient trees and their associated ecosystems, right through to global  
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7 366 biogeochemical processes. There is an urgent need to understand how the genome ~~or~~ hologenome ~~of~~  
8 367 ~~of~~ a microbial community (and in some cases, its host) relates to metabolic capacities. Conversely,  
9  
10 368 there is also a need to understand how ecosystems depend on a particular organism or group of  
11 369 organisms for any given process and function. This section describes the need to move from simply  
12 370 describing microbial diversity to understanding what these organisms are doing, how they are doing it,  
13 371 and what biotic and abiotic drivers are controlling their activity. Each question may derive a suite of  
14 372 different answers, depending on the group of organisms, the habitat and the process.  
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20 374 ~~369~~. What are the mechanisms driving microbial community structure and function, and are these  
21 375 conserved across ecosystems?

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23  
24 376 37. What is the relative importance of stochastic vs. determinative processes in microbial community  
25 377 assembly?

26  
27 378 ~~384~~. How conserved are microbial functions across different spatial and temporal scales?

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29 379 ~~392~~. What is the relative importance of individual 'species' for the functioning of microbial  
30 380 communities?

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33 381 ~~4033~~. How much functional redundancy is there in microbial communities, and how does functional  
34 382 redundancy affect measures of diversity and niche overlap?

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37 383 ~~4134~~. How often are functional traits of microbes successfully conferred through horizontal gene  
38 384 transfer?

39  
40 385 4242. What methods can we use to marry microbial diversity with function; how do we link  
41 386 transcriptomics, proteomics and metabolomics?

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43  
44 387 43. How do we move beyond correlation to develop predictive models that advance our understanding  
45 388 of microbial community function and dynamics?"

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47 389 ~~4450~~. How useful are synthetic communities for testing/infering theories about microbial community  
48 390 dynamics and function?

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395 **Evolutionary Processes**

396 The role of microorganisms in determining evolutionary outcomes of hosts is being investigated in  
397 increasing detail (McFall-Ngai *et al.*, 2013). Experimental evolution studies represent a powerful  
398 means of quantifying host-microbe and microbe-microbe coevolution, and have highlighted the  
399 extraordinary capacity of microbes to act as key mediators of host fitness (e.g. King *et al.* 2016). Whilst  
400 experimental coevolution studies provide a framework for linking dyadic interactions to community-  
401 scale dynamics (Brockhurst & Koskella, 2013), evolutionary principles stemming from macro-ecology  
402 are being applied to microbial communities of humans (Robinson *et al.*, 2010). However, fundamental  
403 biological questions that are well-studied in macrobiology remain controversial for microbial ecology,  
404 for example the species concept remains a source of debate (Freudenstein *et al.* 2016). The  
405 operational taxonomic unit (OTU) has become the standard unit for identifying bacteria at the highest  
406 taxonomic resolution possible, yet it is hard to clearly define where taxonomic boundaries lie between  
407 two bacteria, and what an OTU really represents in biological terms. This is especially problematic in  
408 the context of horizontal gene transfer, which is commonly observed in bacteria and has turned our  
409 understanding of evolutionary processes upside down. This section relates to how general ecological  
410 principles influence microbial evolution and *vice versa*, what this means for global biodiversity, and  
411 whether evolutionary principles can be utilised for anthropogenic gain.

412

413 [4535](#). How can a bacterial 'species' be defined?414 [4636](#). To what extent is faunal and floral biodiversity influenced by microbial communities?415 [4737](#). To what extent do microbial communities have an equivalent to keystone 'species'?416 [4838](#). Does the structure of microbial communities conform to the same ecological rules/principles as  
417 in other types of communities?418 [439](#). How do fundamental shifts in environmental conditions impact the trajectory of microbial  
419 evolution?420 [5040](#). What are the relative selective forces favouring microbial genome expansion or reduction?

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6 421 41. Can experimental evolution predict how antimicrobial resistance evolves *in vivo*?

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10 423 ***Methods in Microbial Ecology***

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12 424 Methods for assessing microbial diversity and community function have rapidly advanced in recent  
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14 425 years, with a major shift from culture dependent to molecular based techniques that produce vast  
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16 426 quantities of data (Rohwer, 2007; Bitten *et al.*, 2015). Advances in technologies for the analysis of  
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18 427 (meta)genomes, (meta)transcriptomes, (meta)proteomes and metabolomes, with associated  
19  
20 428 computational biology tools, have revolutionised our understanding of microbial diversity and function,  
21  
22 429 with multi-'omics' approaches providing unprecedented opportunities to assess genomic potential,  
23  
24 430 gene regulation, expression and functionality *in situ*. There are still many challenges relating to  
25  
26 431 methods for analysing and describing microbiomes, elucidating the roles these microorganisms play  
27  
28 432 both individually and as a community, and how this relates to wider organismal function and  
29  
30 433 environmental processes (Robinson *et al.*, 2010). Amplicon studies also raise the problem of how to  
31  
32 434 link taxonomic identity to functional ability. The vast datasets produced by the 'omics' technologies  
33  
34 435 present unique statistical challenges, requiring new analytical techniques and approaches (Weiss *et*  
35  
36 436 *al.*, 2016ab). Simultaneously, high-throughput culture based methods are being re-invented for  
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38 437 applications such as antibiotic discovery (Ling *et al.*, 2015; Oberhardt *et al.*, 2015). There is no one-  
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40 438 size-fits-all method for a given type of study, but it is desirable to have a suite of robust methods that  
41  
42 439 can be applied in a comparable manner to achieve results with a high level of confidence (Bustin *et*  
43  
44 440 *al.*, 2009). As contemporary technological advancements improve the accessibility, throughput,  
45  
46 441 resolution and cost of microbiome analysis, this section explores some of the new challenges that  
47  
48 442 arise due to rapid advancements within the field, and other research questions that can be addressed.

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45 444 42. What methods can we use to marry microbial diversity with function; how do we link  
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47 445 transcriptomics, proteomics and metabolomics?

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49 446 43. How can we handle the unassigned sequences that dominate metagenome datasets?

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51 447 44. How can we develop a standardized best practice method for analysing sequence data to estimate  
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53 448 relative abundance?

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7 449 ~~45. What is the most appropriate taxonomic level at which to compare microbial community~~  
8 450 ~~compositions?~~  
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10 451 ~~46. How can systems approaches improve our understanding of host-microbe interactions?~~  
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12 452 ~~47. How do we move beyond correlations to determine cause and effect in microbial~~  
13 453 ~~communities/ecosystems?~~  
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16 454 ~~48. How can we better track the source and dispersal of particular microorganisms in real time?~~  
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18 455 ~~49. How can we accurately measure microbial biomass in a reproducible manner?~~  
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20 456 ~~50. How useful are synthetic communities for inferring theories about microbial community dynamics?~~  
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### 23 24 458 **Society and Policy**

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26 459 We need to find ways to apply fundamental biological research to the benefit of society and policy. For  
27 460 example, collaboration with social scientists is crucial when investigating public understanding of  
28 461 microbial ecology, as well as using citizen science approaches to tackle microbial ecology research  
29 462 questions. Many questions relating to this area were discussed at the workshop, and here we present  
30 463 four additional questions that were developed at the meeting that relate to societal and policy-based  
31 464 aspects of microbial ecology.  
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  - How can we best address supply and demand of information about microbial ecology between  
39 467 researchers, clinicians, policy makers and practitioners?
  - How can we best use social and traditional mass media for early identification of emerging  
40 468 threats to animal and plant health?
  - How can we develop an open access data repository or integrate existing databases to create  
41 469 a centralised and standardised method for data and methods sharing in microbial ecology?  
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  - How can we replace fear-based regulation with risk-based regulation, specifically with regard  
43 471 to the use of microbes in bioremediation and bioaugmentation?  
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475 **Discussion**

476 Here we present 50 important research questions across eight a number of themes relating to the field  
477 of microbial ecology. Although there are many other research issues worthy of investigation, it is  
478 intended that these questions will be used to inform and direct future research programmes and  
479 agendas, particularly in areas where microbial ecology has not previously been considered or applied.  
480 In many cases, these questions are deliberately broad to allow researchers to adapt them to their own  
481 areas of interest, for example across different systems, or to varying spatial scales. Across many  
482 questions there was strong recognition of the vast metabolic capabilities of microorganisms and  
483 microbial communities, and the need to harness this power to improve human and animal health and  
484 wellbeing. Some themes addressed various existing mechanisms for exploiting microbial processes,  
485 namely bioremediation, soil improvement, water treatment and probiotic suppression of pathogen  
486 resistance. As these are already active areas of research, the questions posed here are structured to  
487 provide a framework by which these efforts can be directed in the future.

488 A predominant theme that emerged was the need to integrate knowledge between different  
489 research areas, for example the application of information from human microbiome studies to the  
490 study of other non-model host organisms, and the potential to apply macro-ecological frameworks to  
491 micro-ecological concepts. Many fundamental biological questions that are well-studied in classical  
492 ecology remain controversial for microbial ecology, and the species concept (Freudenstein *et al.*  
493 2016), taxonomy, and how the OTU should be defined for microorganisms, generated multiple  
494 questions (e.g. see '*Evolutionary Processes*' theme). Classical community ecology concepts should  
495 not be overlooked when considering microbial dynamics (Rynkiewicz *et al.*, 2015) and, conversely,  
496 microbial communities may prove useful models for general ecology due to their short generation  
497 times, reproducibility, and ease of use in the laboratory environment (Brockhurst & Koskella, 2013;  
498 Libberton *et al.*, 2015; King *et al.*, 2016). There have been a number of calls for the medical profession  
499 to look to ecological and evolutionary tools when seeking to understand epidemiology (Johnson *et al.*,  
500 2015), investigating novel antibacterial agents (Vale *et al.*, 2016), and considering multi-host, multi-  
501 agent disease systems (Buhnerkempe *et al.*, 2015).

502 The '*Host-Microbiome Interactions*' theme considered the need to understand factors  
503 influencing microbiome composition, which in turn have consequences for a myriad of host traits,  
504 including disease susceptibility and host evolution (Chisholm *et al.*, 2006; Archie & Theis, 2011; Spor

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7 505 *et al.*, 2011; Cho & Blaser, 2012; McFall-Ngai *et al.*, 2013; McFall-Ngai, 2015; Zilber-Rosenberg &  
8 506 Rosenberg, 2008). As this theme considered microbiota from the perspective of the host, there was  
9  
10 507 some overlap with the '*Health and Infectious Diseases*' and '*Evolutionary Processes*' themes.  
11 508 Probiotics were discussed as a viable and promising alternative to current strategies in a number of  
12  
13 509 contexts in these themes, not only to improve individual health, but also to decrease disease  
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15 510 susceptibility of humans and other animals, to enhance nutritional quality of food, and to mitigate the  
16 511 negative impacts of antibiotic use across humans, livestock, aquaculture and agriculture (Martín *et al.*,  
17 512 2013; Newaj-Fyzul *et al.*, 2014; Smith, 2014; Fox, 2015). Developing personalized probiotic-based  
18 513 therapies requires complementary diversity and functional-based studies in order to elucidate the  
19 514 specific roles of microbiota in health and disease, and thus how microbial communities can be  
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21 515 manipulated.

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25 516 Likewise, qQuestions considered in both the '*Functional Diversity*' theme and the  
26 517 '*Environmental Processes*' theme raised a common need to understand changes in microbial  
27 518 community structure and function across spatial and temporal scales (Carmona *et al.*, 2016).  
28 519 Establishing appropriate spatial scales for studying microbial processes is an outstanding challenge:  
29 520 micro-organisms can orchestrate ecosystem functioning across whole biomes (Sheffer *et al.*, 2015),  
30 521 yet fungi exhibit low mobility on tree barks (Koufopanou *et al.* 2006, Robinson *et al.*, 2016), and an air  
31 522 void in soil can be an insuperable barrier for a bacterium. Similarly, drawing meaningful conclusions  
32 523 about microbial processes requires understanding of their temporal variability; for exampleexample,  
33 524 diurnal influences (Shurpali *et al.*, 2016), or lags behind changes in ecosystem drivers (Allison and  
34 525 Martiny, 2008). These concerns were ultimately addressed in a single question (question 28).

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41 526 Questions identified in this paper highlighted the need for knowledge that  
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43 527 informs antibiotic use and production, and to optimise the sustainability of food  
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45 528 production through improved soil fertility. There was a strong interest in maintaining  
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47 529 and enhancing the microbial populations of crop ecosystems, especially in light of  
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49 530 antibiotic resistance (Elouze *et al.*, 2014). As antibiotic resistance increases along with  
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51 531 our concern about potential impact on both human and animal health, there is an  
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53 532 increasing drive to find new forms of antibiotics.

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7 533 Probiotics were discussed as a viable and promising alternative (Martin et al., 2013; Nowaj-  
8 534 Fyzul et al., 2014; Smith, 2014; Fox, 2015), not only to improve individual health, but also to decrease  
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10 535 disease susceptibility, enhance nutritional quality of food, and to mitigate the negative impacts of  
11 536 antibiotic use across humans, livestock, aquaculture and agriculture (Martin et al., 2013; Nowaj Fyzul  
12 537 et al., 2014; Smith, 2014; Fox, 2015). Developing personalized probiotic-based therapies requires  
13 538 complementary diversity and functional-based studies in order to elucidate the specific roles of  
14 539 microbiota in health and disease, and thus how microbial communities can be manipulated.

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16 540 Global changes resulting from human activity impact almost every Earth habitat. It is  
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18 541 imperative that we focus efforts on understanding the impacts of human activities such as climate  
19 542 change, urbanisation, agriculture, and industrial processes on microbial communities, ecosystem  
20 543 functioning equilibrium, and host health. Microbial populations have a tremendous capacity to adapt to  
21 544 changes in their abiotic environment, yet the functional implications of these transitions in microbial  
22 545 ecology are still poorly understood and characterised (Bissett et al., 2013), and the role of microbes in  
23 546 mediating the response of larger organisms to change is equally understudied. Plant-associated  
24 547 microbial communities can alter the performance of subsequent generations of plants, and can  
25 548 themselves be subject to the concomitant effects of abiotic change (van der Putten et al., 2013). Such  
26 549 plant-soil feedbacks can reduce yield and alter the community composition and invasibility of whole  
27 550 areas of grassland (van der Putten et al., 2013). Discussions resulting from the 'Functional Diversity'  
28 551 theme (questions 30-34) addressed the need to move from simply describing microbial diversity to  
29 552 understanding what organisms are doing, how they are doing it, and which biotic and abiotic drivers  
30 553 control this activity. Each of these questions will likely derive a suite of different answers, depending  
31 554 on the group of organisms, the habitat and the process; the questions presented in this section were  
32 555 therefore some of the broadest discussed.

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34 556 Large-scale assessments of ecosystem services and degradation acknowledge the paucity of  
35 557 data on microbial impacts, presumably because there are no convincing large-scale messages that  
36 558 can be derived at this stage (Norris et al., 2011). Microbial diversity is therefore rarely considered  
37 559 when estimates of biodiversity are required for policy or management decisions. Microbial diversity,  
38 560 abundance and influence on the biodegradation of important naturally produced atmospheric  
39 561 hydrocarbons such as isoprene, have recently been studied to determine which micro-organisms are  
40 562 important in the sink of this global climate altering gas (Dumont & Murrell, 2015; El Khawand et al.



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7 563 | ~~2016). Another key greenhouse gas that needs further study is methane, the flux of which is regulated~~  
8 564 | ~~by methanotrophic bacteria and methanogenic Archaea, and can substantially alter the carbon~~  
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10 565 | ~~balance of a system and have grave consequences for global climate change (e.g. Schuur *et al.* 2015;~~  
11 566 | ~~James *et al.* 2016). Solving these types of questions will help us to understand the impact of human~~  
12 567 | ~~activities on microbial ecology, and to find new solutions to the environmental and health problems we~~  
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14 568 | ~~are currently facing and will continue to face in the future.~~

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17 569 | A subject common to a number of themes was the role of individual species *versus* consortia  
18 570 | in ~~ecosystem community~~ functioning. The question of defining bacterial species is a contentious topic,  
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20 571 | and the issue remains whether some microbial taxa act as keystones in ecosystem functions. Many  
21 572 | microbial surveys carry the implicit assumption that the most abundant taxa are also the most  
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23 573 | important, yet rare species can be hugely significant if they are highly active and/or monopolise a  
24 574 | particular process (Lynch and Neufeld, 2015). The collective metabolic capabilities of micro-organisms  
25 575 | have great potential for *in situ* applications such as bioremediation, particularly when used in multi-  
26 576 | species consortia (Mikesková *et al.*, 2012). Successful bioremediation and environmental  
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28 577 | management requires the introduction of new assemblages ~~wholesale~~ into an established community,  
29 578 | or stimulation of key members of the community *in situ* (Rillig *et al.*, 2015). In turn, predicting the  
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31 579 | successful establishment of deliberately introduced organisms depends on an understanding of the  
32 580 | principles underlying microbial community formation and structure (Rillig *et al.*, 2015). Despite these  
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34 581 | challenges, functional diversity modelling has successfully been applied to the ecological restoration of  
35 582 | some plant communities (Laughlin, 2014). Closely linked to this is the issue of functional redundancy,  
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37 583 | and to what extent it is possible to lose species without affecting ecosystem functions. Already there is  
38 584 | evidence that microbial communities may be less functionally redundant than macro-organism  
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40 585 | communities (Delgado-Baquerizo *et al.*, 2016). This issue ties into fundamental ecological concepts,  
41 586 | such as niche theory (Carmona *et al.*, 2016); if multiple organisms are carrying out the same process,  
42  
43 587 | apparently interchangeably, how do they avoid competitively excluding one another? The concept of  
44 588 | keystone species has been shown to be applicable to microbes (Neufeld *et al.*, 2008; Pester *et al.*,  
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46 589 | 2010; Ze *et al.*, 2012; Yu *et al.*, 2016), yet further work is needed to characterise the extent to which  
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48 590 | keystone functions occur in different environments and whether these can be consistently identified  
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50 591 | (Anderson, 2003; Pester *et al.*, 2010).

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52 592 | ~~Recent major technological and analytical advancements have made previously inaccessible~~  
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7 593 ~~taxa and ecosystems amenable to study. Despite the opportunities afforded by big data however,~~  
8 594 ~~there are huge challenges when handling next generation sequencing data, both in the size of the~~  
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10 595 ~~datasets and the spurious inter-correlation (compositionality) inherent to the sequencing process~~  
11 596 ~~(Weiss *et al.*, 2016a). There is community wide recognition that current methods of analysis are not~~  
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13 597 ~~adequate to deal with the big data produced by next-generation sequencing, and a number of groups~~  
14 598 ~~have already attempted to establish standardised analysis methods (e.g. amplicon sequencing of~~  
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16 599 ~~bacterial and fungal communities (Thomas *et al.*, 2012; Smith & Peay, 2014), and bioinformatics of~~  
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18 600 ~~targeted and shotgun metagenomics (Bokulich *et al.*, 2013; McMurdie & Holmes, 2014; Oulas *et al.*,~~  
19  
20 601 ~~2015; Randle-Boggis *et al.*, 2016). These methods now require validation under different contexts to~~  
21 602 ~~keep analyses with similar aims and methods comparable (Weiss *et al.*, 2016a,b). This is no easy task~~  
22  
23 603 ~~given the plethora of decisions made throughout research design, from sample collection and storage~~  
24 604 ~~to lab work and data analysis (Callahan *et al.*, 2016), each of which introduce complexity to a study's~~  
25  
26 605 ~~approach.~~

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28 606       The need for open access databases and repositories, both in the context of data sharing as  
29  
30 607 well as for methods and protocols, was reflected in the questions shortlisted for the '*Society and*  
31 608 *Policy*' theme. Discussions included the benefits of forming collaborative and open research  
32  
33 609 communities, and the need to ensure the legacy of academic research through improving regulation  
34 610 and policy and engagement with the public. Fear-based regulation of research, grounded in alarmist or  
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36 611 populist campaigns, as opposed to risk-based regulation built upon evidence, was identified as a  
37 612 possible obstacle to progress, which could be addressed through greater interaction between  
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39 613 microbial ecologists and the public at both governmental and grass roots levels. Large scale  
40 614 assessments of ecosystem services and degradation acknowledge the paucity of data on microbial  
41 615 impacts, presumably because there are no convincing large-scale messages that can be derived at  
42 616 this stage (Norris *et al.*, 2011). Microbial diversity is therefore rarely considered when estimates of  
43 617 biodiversity are required for policy or management decisions. That said, ~~the~~ increasing recognition of  
44 618 the fundamental impact of the microbial world on the functioning of larger-scale processes has made  
45  
46 619 the deliberate manipulation of the microbial world a controversial subject, which was reflected in the  
47 620 number of draft questions submitted related to bioremediation and bioaugmentation (see  
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49 621 Supplementary Information). Collaboration with social scientists was identified as crucial in gauging  
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51 622 the public understanding of microbial ecology, and citizen science approaches were considered as

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623 tools to tackle key microbial ecology research questions. ▲

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624 The 50 questions identified here cover a broad range of topics, but some over-arching themes  
625 recur across multiple questions, including a recognition that microbes play an important role in a  
626 variety of different processes and systems, which may be harnessed to solve real-world problems.

627 There were some similarities between the questions identified here and those identified by previous  
628 workshops of a similar nature. For example, questions relating to soil health and biodiversity (Dicks *et*  
629 *al.* 2013), a requirement for developing a theoretical understanding of micro- and macro- ecological  
630 concepts (Prosser *et al.* 2007, Sutherland *et al.* 2013a) and disease dynamics (Prosser *et al.* 2007,

631 Sutherland *et al.* 2013a) have a degree of commonality with this list. This indicates that the ecological

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632 theory underpinning many research questions transcends scientific disciplines, and that there is still  
633 much work to be done at both theoretical and applied levels. Within these 50 questions, we have tried  
634 to provide a focus for researchers addressing scientific questions from a microbial perspective,

635 regardless of their background. It is expected that these questions will facilitate interesting discussion

636 and new, exciting, interdisciplinary research. The list is by no means exhaustive, and we recognise

637 that the questions presented here are relatively community-centric, primarily due to the recent

638 expansion in methodological approaches that have improved our understanding of microbial

639 community diversity and function. That said, other areas of microbial ecology should not be ignored or

640 forgotten. and given the rapidly evolving field of microbial ecology, it is expected that future

641 workshops with a wide draw will be held to ensure that the identification of research priorities and

642 areas of interest is a continuing process.

643

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650 Safety Authority.

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10 654 questions, and to Jessica Hall, Daniel Henk and Matt Lloyd Jones for their contributions to the  
11 655 workshop.  
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16 657 **References**

- 17  
18 658 Allison, S, Martiny, J Resistance, resilience, and redundancy in microbial communities. *PNAS*,  
19 659 2008;**105 S1**, 11512-11519.  
20  
21 660 Anderson, TH Microbial eco-physiological indicators to asses soil quality. *Agric Ecosyst Environ*,  
22 661 2003;**98**, 285–293  
23  
24 662 Archie, EA, Theis, KR Animal behaviour meets microbial ecology. *Anim Behav*, 2011;**82**, 425-436  
25  
26 663 Baothman, OA, Zamzami, MA, Taher, I *et al*. The role of Gut Microbiota in the development of obesity  
27 664 and Diabetes. *Lipids Health Dis*, 2016;**15**, 108.  
28  
29 665 Bissett, A, Brown, MV, Siciliano, SD *et al*. Microbial community responses to anthropogenically  
30 666 induced environmental change: towards a systems approach. *Ecol Lett*, 2013;**16**, 128-139.  
31  
32 667 Boers, SA, Jansen, R, Hays, JP Suddenly everyone is a microbiota specialist. *Clin Microbiol Infect*,  
33 668 2016;**22**, 581-2.  
34  
35 669 Bokulich, NA, Subramanian, S, Faith, JJ, *et al*. Quality-filtering vastly improves diversity estimates  
36 670 from Illumina amplicon sequencing. *Nat Methods*, 2013;**10**, 57-59  
37  
38 671 Bragazza, L, Parisod, J, Buttler, A, *et al*. Biogeochemical plat-soil microbe feedback in response to  
39 672 climate warmings in peatlands. *Nat. Clim. Chang*, 2013;**3**, 273-277.  
40  
41 673 Brockhurst MA, Koskella B Experimental coevolution of species interactions. *Trends Ecol Evol*,  
42 674 2013;**28**, 367–375  
43  
44 675 Brown, S, Veach, A, Rigdon-Huss, A, *et al*. Scraping the bottom of the barrel: are rare high throughput  
45 676 sequences artifacts? *Fungal Ecol*, 2015;**13**, 221-225  
46  
47 677 Buhnerkempe, MG, Roberts, MG, Dobson, AP, *et al*. Eight challenges in modelling disease ecology in  
48 678 multi-host, multi-agent systems. *Epidemics*, 2015;**10**, 26-30  
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60

- 679 Buonauro, R, Moretti, C, Passos da Silva, D, *et al.* The olive knot disease as a model to study the role  
680 of interspecies bacterial communities in plant disease. *Front. Plant Sci*, 2015;**6**: 434.
- 681 Bustin, S, Benes, V, Garson, J, *et al.* The MIQE guidelines: minimum information for publication of  
682 quantitative real-time PCR experiments. *Clin. Chem*, 2009;**55**, 611-22
- 683 Callahan, B, Proctor, D, Relam, D, *et al.* Reproducible research workflow in R for the analysis of  
684 personalised human microbiome data. *Pacific Symposium on Biocomputing*, 2016;**21**, 183-194.
- 685 Carmona, C, de Bello, F, Mason, N, *et al.* Traits Without Borders: Integrating Functional Diversity  
686 Across Scales. *TREE*, 2016;**31**, 382-394
- 687 Chaparro, JM., Sheflin, AM, Manter, DK, *et al.* Manipulating the soil microbiome to increase soil health  
688 and plant fertility. *Biol Ferti Soils*, 2012;**48**, 489-499
- 689 Chibuike, GU, Obiora, SC Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods.  
690 *App Env Soil Sci*, 2014; Article ID 752708 1-12
- 691 Chin, JP, McGrath, JW, Quinn, JP. Microbial transformations in phosphate biosynthesis and  
692 catabolism, and their importance in nutrient cycling. *Current Opinion in Chemical Biology*, 2016;**31**, 50-  
693 57
- 694 Chisholm, ST, Coaker, G, Day, B, *et al.* Host-microbe interactions: Shaping the evolution of plant  
695 immune response. *Cell* 2006;**126**, 803-814
- 696 Cho, I, Blaser, MJ. The human microbiome: at the interface of health and disease. *National Review of*  
697 *Genetics*, 2013;**13**, 260-270
- 698 Coker, JA Extremophiles and biology: current uses and prospects. *F1000 Research*, 2016;**5**, F1000  
699 FacultyRev-396
- 700 Creamer, CA, de Menezes, AB, Krull, ES, *et al.* Microbial community structure mediates response of  
701 soil C decomposition to litter addition and warming. *Soil Biol Biochem*, 2015;**80**, 175-188
- 702 Daskin, JH, Alford, RA Context-dependent symbioses and their potential roles in wildlife diseases.  
703 *Proc Bio Sci*. 2012;**279**, 1457-65
- 704 Delgado-Baquerizo, M, Giaramida, L, Reich, P, *et al.* Lack of functional redundancy in the relationship  
705 between microbial diversity and ecosystem functioning. *J. Ecol*, 2016;**104**, 936-946

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2  
3  
4  
5  
6  
7 706 Dicks, LV, Abrahams, A, Atkinson, J, *et al.* Identifying key knowledge needs for evidence-based  
8 707 conservation of wild insect pollinators: a collaborative cross-sectoral exercise. *Insect Conservation*  
9 708 *and Diversity*, 2012;**6**, 435-446
- 11 709 Dicks, LV, Bardgett, RD, Bell, J, *et al.* What do we need to know to enhance the environmental  
13 710 sustainability of agriculture? A prioritisation of knowledge needs for the UK food system. *Sustainability*,  
14 711 2013;**5**, 3095–3115
- 16 712 Dumont, MG, Murrell, JC. Community-level analysis: key genes of aerobic methane oxidation.  
18 713 *Methods Enzymol*, 2015;**397**, 413-427.
- 20 714 El Khawand, M, Crombie, AT, Johnston, A, *et al.* Isolation of isoprene degrading bacteria from soils,  
22 715 development of isoA gene probes and identification of the active isoprene degrading soil community  
23 716 using DNA-stable isotope probing. *Environ Microbiol*, 2016;**18**, 2743-53
- 25 717 Ellouze, W, Esmaili-Taheri, A, Bainard, LD, *et al.* Soil Fungal Resources in Annual Cropping Systems  
27 718 and Their Potential for Management. *BioMed Research International*, 2014;531824
- 29 719 Freudenstein, JV, Broe, MB, Folk, *et al.* Biodiversity and the species concept-Lineages are not  
31 720 enough. *Systematic Biology*, 2016;10.1093
- 33 721 Fox, JL. Agricultural probiotics enter spotlight. *Nature Biotechnology*, 2015;**33**, 122
- 35 722 Franklin AM, Aga, DS, Cytryn, E, *et al.* Antibiotics in agroecosystems: Introduction to the special  
36 723 section. *J. Env Qual*, 2016;**45**, 377
- 38 724 Franzosa, E, Hsu, T, Sirota-Madi, A, *et al.* Sequencing and beyond: integrating molecular 'omics' for  
40 725 microbial community profiling. *Nat. Rev. Microbiol*, 2015;**13**, 360-372
- 42 726 Fredriksson, N, Hermansson, M, Wilén, B The choice of PCR primers has great impact on  
44 727 assessments of bacterial community diversity and dynamics in a wastewater treatment plant. *PLoS*  
45 728 *One*, 2013;**8**, e76431
- 47 729 Haritash, AK., Kaushik, CP Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A  
49 730 review. *J Hazard Mater*, 2009;**169**, 1-15
- 51 731 Hartmann, M, Frey, B, Mayer, J, *et al.* Distinct soil microbial diversity under long-term organic and  
52 732 conventional farming. *ISME J*, 2015;**9**, 1177-1194

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60

733 Helgason, T, Daniell, TJ, Husband, R, *et al.* Ploughing up the wood-wide web? *Nature*, 1998;**394**, 431-  
734 431

735 Hiraoka, S, Yang, CC, Iwasaki, W Metagenomics and bioinformatics in microbial ecology: current  
736 status and beyond. *Microbes and Environments*, 2016;**31**, 204-212

737 Hu, HW, Chen, D, He, JZ Microbial regulation of terrestrial nitrous oxide formation: understanding the  
738 biological pathways for prediction of emission rates. *FEMS Microbiol Rev*, 2015;**39**, 729-749

739 Jacobsen, CS, Hjelmso, MH. Agricultural soils, pesticides and microbial diversity. *Current opinion in*  
740 *biotechnology*, 2014;**27**, 15-20

741 Johnson, PT, de Roode, JC, Fenton, A (2015). Why infectious disease research needs community  
742 ecology. *Science*, 349, 1259504

743 James, R. H., Bousquet, P., Bussmann, I., Haeckel, M., Kipfer, R., Leifer, I., Niemann, H., Ostrovsky,  
744 I., Piskozub, J., Rehder, G., Treude, T., Vielstädt, L. and Greinert, J. Effects of climate change on  
745 methane emissions from seafloor sediments in the Arctic Ocean: A review. *Limnol. Oceanogr.*, 2016;  
746 **61**: S283–S299. doi:10.1002/lno.10307

747 Jones, AC, Mead, A, Kaiser, MJ, *et al.* Prioritization of knowledge needs for sustainable aquaculture: a  
748 national and global perspective. *Fish and Fisheries*, 2014;**16**, 668–683.

749 Koch, H, Schmid-Hempel, P Socially transmitted gut microbiota protect bumble bees against an  
750 intestinal parasite. *Proc Natl Acad Sci USA*, 2011;**108**, 19288–19292

751 Koufopanou, V, Hughes, J, Bell, G, *et al.* The spatial scale of genetic variation in a model organism:  
752 the wild yeast *Saccharomyces paradoxus*. *Phil Trans B* 2006;**29**, 1941-1946

753 King, KC, Brockhurst, MA, Vasieva, O, *et al.* Rapid evolution of microbe-mediated protection against  
754 pathogens in a worm host. *ISME J*, 2016;10.1038/ismej.2015.259

755 Krishnasamy V, Otte J, Silbergeld E Antimicrobial use in Chinese swine and broiler poultry production.  
756 *Antimicrob Resist Infect Control*, 2015;**4**, 17.

757 Levy, SB Antibiotic resistance: an ecological imbalance. *Antibiotic resistance: Origins, Evolution,*  
758 *Selection and Spread*. Wiley, Chichester: Ciba Foundation Symposium, 1997;**207**, 1-17



- 1  
2  
3  
4  
5  
6  
7 759 Ley, RE, Hamady, M, Lozupone, C, *et al.* Evolution of mammals and their gut microbes. *Science*,  
8 760 2008;**320**, 1647–1651  
9  
10 761 Libberton, B, Horsburgh, MJ, Brockhurst, MA The effects of spatial structure, frequency dependence  
11 762 and resistance evolution on the dynamics of toxin-mediated microbial invasions. *Evol. App*, 2015;**7**,  
12 763 738-750  
13  
14  
15 764 Ling, L, Schneider, T, Peoples, T, *et al.* A new antibiotic kills pathogens without detectable resistance.  
16 765 *Nature*, 2015;**517**, 455–459  
17  
18  
19 766 Lozupone, CA., Li, M, Campbell, TB, *et al.* Alterations in the gut microbiota associated with HIV-1  
20 767 infection. *Cell host & microbe*, 2013;**14**, 329-339  
21  
22  
23 768 Lynch, M, Neufeld, J Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol*, 2015;**13**,  
24 769 217–229  
25  
26 770 Martín, M, Miquel, S, Ulmer, J, *et al.* Role of commensal and probiotic bacteria in human health: a  
27 771 focus on inflammatory bowel disease. *Microbial Cell Factories*, 2013;**12**, 71  
28  
29  
30 772 McFall-Ngai, M, Hadeldb, MG, Boschc, TCG, *et al.* Animals in a bacterial world, a new imperative for  
31 773 the life sciences. *P Natl Acad Sci USA*, 2013;**110**, 3229-3236  
32  
33  
34 774 McFall-Ngai, MJ. Giving microbes their due--animal life in a microbially dominant world. *J Exp Biol*,  
35 775 2015;**218**, 1968-1973  
36  
37 776 McMurdie, PJ, Holmes, S Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS*  
38 777 *Comput Biol*, 2014;**10**, e1003531  
39  
40  
41 778 Morris, CE, Bardin, M, Berge, O, *et al.* Microbial biodiversity: approaches to experimental design and  
42 779 hypothesis testing in primary scientific literature from 1975 to 1999. *Micr Mol Biol Rev*, 2002;**66**, 592-  
43 780 616  
44  
45  
46 781 Mueller, K, Ash, C, Pennisi, E, *et al.* The gut microbiota. *Science*, 2012;**336**, 1245  
47  
48 782 Newaj-Fyzul, A, Al-Harbi, H, Austin, B Review: Developments in the use of probiotics for disease  
49 783 control in aquaculture. *Aquaculture*, 2014;**431**, 1-11  
50  
51  
52 784 Neufeld, JD, Chen, Y, Dumont, MG, *et al.* Marine methylotrophs revealed by stable-isotope probing,  
53 785 multiple displacement amplification and metagenomics. *Environ Microbiol*, 2008;**10**, 1526–35  
54  
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786 Norris, K, Bailey, M, Baker, S, *et al.* Biodiversity in the context of ecosystem services. *In: The UK*  
787 *National Ecosystem Assessment Technical Report. UK National Ecosystem Assessment, UNEP-*  
788 *WCMC, Cambridge. 2011*

789 Oberhardt, M, Zarecki, R, Gronow, S, *et al.* Harnessing the landscape of microbial culture media to  
790 predict new organism–media pairings. *Nat. Comm*, 2015;**6**, 8493

791 Oller, I, Malato, S, Sanchez-Perez, JA Combination of advanced oxidation processes and biological  
792 treatment for wastewater decontamination- a review. *Sci Total Environ*, 2011;**409**, 4141-4166.

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793 Oulas, A, Pavloudi, C, Polymenakou, P, *et al.* Metagenomics: tools and insights for analysing next-  
794 generation sequencing data derived from biodiversity studies. *Bioinform Biol Insights*, 2-15;**9**, 75-88

795 Parsons, ECM, Favero, B, Aguirre, AA, *et al.* Seventy-one important questions for conservation of  
796 marine biodiversity. *Conserv Biol*, 2014;**28**, 1206–1214

797 Peay, K Back to the future: natural history and the way forward in modern fungal ecology. *Fungal Ecol*,  
798 2014;**2**, 4-9

799 Pester, M, Bittner, N, Deevong, P, *et al.* A 'rare biosphere' microorganism contributes to sulfate  
800 reduction in a peatland. *ISME J*, 2010;**4**, 1591–602

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801 Prosser, JI, Bohannan, BJM, Curtis, TP, *et al.* The role of ecological theory in microbial ecology.  
802 *Nature*, 2007;**5**, 384-392

803 Randle-Boggis, RJ, Helgason, T, Sapp, M, *et al.* Evaluating techniques for metagenome annotation  
804 using simulated sequence data. *FEMS Mic Ecol*, 2016;**92**,

805 Rebollar, EA, Antwis, RE, Becker, MH, *et al.* Using "Omics" and Integrated Multi-Omics Approaches to  
806 Guide Probiotic Selection to Mitigate Chytridiomycosis and Other Emerging Infectious Diseases. *Front*

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807 *Microbiol*, 2016;**7**, 68

808 Redford, KH, Segre, JA, Salafsky, N, *et al.* Conservation and the microbiome. *Conserv Biol*, 2012;**26**,  
809 195-197

810 Ridaura, VK, Faith, JJ, Rey, FE, *et al.* Gut Microbiota from Twins Discordant for Obesity Modulate  
811 Metabolism in Mice. *Science*, 2013;**341**, 1241214

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- 1  
2  
3  
4  
5  
6  
7 812 Robinson, CJ, Bohannan, BJ, Young, VB From structure to function: the ecology of host-associated  
8 813 microbial communities. *Microbiol Mol Biol Rev*, 2010;**74**, 453-476  
9  
10 814 Robinson, HA, Pinharanda, A, Bensasson, DB. Summer temperature can predict the distribution of  
11 815 wild yeast populations. *Ecol Evol*, 2016;**27**, 1236-1250  
12  
13  
14 816 Round, JL, Mazmanian, SK. The gut microbiota shapes intestinal immune responses during health  
15 817 and disease. *Nat Rev Immunol*, 2009;**9**, 313-323  
16  
17 818 Rynkiewicz, EC, Pedersen, A.B., Fenton, A An ecosystem approach to understanding and managing  
18 819 within-host parasite community dynamics. *Trends in Parasitology*, 2015;**31**, 212-221  
19  
20  
21 820 Sato, Y, Civiello, M, Bell, S, *et al*. Intergrated approach to understanding the onset and pathogenesis  
22 821 of black band disease in corals. *Environmental Microbiology*, 2016;**18**, 752-765  
23  
24  
25 822 Schneider, T, Keiblinger, K, Schmid, E, *et al*. Who is who in litter decomposition? Metaproteomics  
26 823 reveals major microbial players and their biogeochemical functions. *ISME J*, 2012;**6**, 1749-1762  
27  
28 824 Schuur, EAG, McGuire, AD, Schädel, C *et al*. Climate change and the permafrost carbon feedback.  
29 825 *Nature*, 2015;**520**, 171-179  
30  
31  
32 826 Serino, M, Nicholas, M, Trabelsi, MS, *et al*. Young microbes for adult obesity. *Pediatric Obesity*,  
33 827 2016;**10**.1111.Sheffer, E, Batterman, S, Levin, S, *et al*. Biome-scale nitrogen fixation strategies  
34 828 selected by climatic constraints on nitrogen cycle. *Nat Plants*, 2015;**1**, 15182  
35  
36  
37 829 Shurpali, N, Rannik, U, Jokinen, S, *et al*. Neglecting diurnal variations leads to uncertainties in  
38 830 terrestrial nitrous oxide emissions. *Sci Rep*, 2016;**6**, 25739  
39  
40  
41 831 Singh, BK., Bardgett, RD., Smith, P, *et al*. Microorganisms and climate change: terrestrial feedbacks  
42 832 and mitigation options. *Nat Rev Microbiol*, 2010;**8**, 779-790  
43  
44 833 Smith, JMA Review of Avian Probiotics, *Journal of Avian Med Surg*, 2014;**28**, 87-94  
45  
46 834 Smith, DP, Peay, KG Sequence depth, not PCR replication, improves ecological inference from next  
47 835 generation sequencing. *PLoS One*, 2014;**9**, e09234  
48  
49  
50 836 Smits, HH, Hiemstra, PS, Prazeres da Costa, C, *et al*. Microbes and asthma: Opportunities for  
51 837 intervention. *J Allergy Clin Immunol*, 2016;**137**(3), 690-697  
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- 838 Spor, A, Koren, O, Ley, R Unravelling the effects of the environment and host genotype on the gut  
839 microbiome. *Nat Rev Microbiol*, 2011;**9**, 279-290
- 840 Sutherland, WJ, Adams, WM, Aronson, RB, *et al.* One hundred questions of importance to the  
841 conservation of global biological diversity. *ConservBiol*, 2009;**23**, 557-567
- 842 Sutherland, WJ., Fleishman, E, Mascia, MB, *et al.* Methods for collaboratively identifying research  
843 priorities and emerging issues in science and policy. *MEE*, 2011;**2**, 238-247
- 844 Sutherland, WJ, Armstrong-Brown, S, Armsworth, PR, *et al.* Identification of 100 fundamental  
845 ecological questions. *J Ecol*, 2013a;**101**, 58–67
- 846 Sutherland, WJ, Goulden, C, Bell, K, *et al.* 100 Questions: Identifying research priorities for poverty  
847 prevention and reduction. *J Poverty Soc Justice*, 2013b;**21**, 189–205
- 848 Thomas, V, McDonnell, G, Denyer, SP, *et al.* Free-living amoebae and their intracellular pathogenic  
849 microorganisms: risks for water quality. *FEMS Microbiol Rev*, 2010;**34**, 231-259
- 850 Thomas, T, Gilbert, J, Meyer, F Metagenomics – a guide from sampling to data analysis. *Microb*  
851 *Inform Exp*, 2012;**2**, 3
- 852 Ubeda, C, Bucci, V, Caballero, S, *et al.* Intestinal microbiota containing *Barnesiella* species cures  
853 vancomycin-resistant *Enterococcus faecium* colonization. *Infect Immun*, 2013;**81**, 965-973.
- 854 Vale, PF., McNally, L, Doeschl-Wilson, A, *et al.* Beyond Killing: Can we find new ways to manage  
855 infection? *Evol Med Public Health*, 2016; doi: 10.1093/emph/eow012
- 856 ~~van der Putten, WH, Bardgett, RD, Bever, JD, *et al.* Plant-soil feedbacks: the past, the present and~~  
857 ~~future challenges. *J Ecol*, 2013 :**101**, 265–276~~
- 858 Vayssier-Taussat M, Albina E, Citti C, *et al.* Shifting the paradigm from pathogens to pathobiome: new  
859 concepts in the light of meta-omics. *Front Cell Infect Microbiol*, 2014;**4**, 29
- 860 Walter, J, Ley, R The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev*  
861 *Microbiol*, 2011;**65**, 411-429
- 862 Weiss, B, Aksoy, S. Microbiome influences on insect host vector competence. *Trends in Parasitology*,  
863 2011;**27**, 514–522

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4  
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6  
7 864 Weiss, S, Van Treuren, W, Lozupone, C, *et al.* Correlation detection strategies in microbial data sets  
8 865 vary widely in sensitivity and precision. *ISME J*, 2016a;**10**, 1669–1681  
9  
10 866 Weiss, S, Xu, Z, Amir, A, *et al.* Effects of library size variance, sparsity, and compositionality on the  
11 867 analysis of microbiome data. *Peer J*, 2016b;**3**, e1408  
12  
13  
14 868 WHO. Tackling Antibiotic Resistance from a Food Safety Perspective in Europe. Copenhagen,  
15 869 Denmark: WHO Regional Office for Europe 2011. Available:  
16  
17 870 <http://www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety->  
18 871 [perspective-in-europe](http://www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety-) [accessed 19 July 2016].  
19  
20 872 Widder, S, Widder, S, Allen, RJ *et al.* Challenges in microbial ecology: building predictive  
21 873 understanding of community function and dynamics. *ISME J*, 2016;DOI: 10.1038/ismej.2016.45  
22  
23  
24 874 Wieder, WR, Bonan, GB, Allison, SD Global soil carbon projections are improved by modelling  
25 875 microbial processes. *Nat Clim Change*, 2013;**3**, 909-912  
26  
27  
28 876 Willing, BP, Russell, SL, Finlay, BB Shifting the balance: antibiotic effects on host–microbiota  
29 877 mutualism. *Nat Rev Microbiol*, 2011;**9**, 233-243  
30  
31 878 Yu, Z, Krause, SMB, Beck, DAC, *et al.* Synthetic Ecology Perspective: How Well Does Behavior of  
32 879 Model Organisms in the Laboratory Predict Microbial Activities in Natural Habitats? *Front Microbiol*,  
33 880 2016;**7**, 1–7  
34  
35  
36  
37 881 Ze, X, Duncan, SH, Louis, P, *et al.* *Ruminococcus bromii* is a keystone species for the degradation of  
38 882 resistant starch in the human colon. *ISME J*, 2012;**6**, 1535–1543  
39  
40 883 Zilber-Rosenberg, I, Rosenberg, E Role of microorganisms in the evolution of animals and plants: the  
41 884 hologenome theory of evolution. *FEMS Micro Rev*, 2008;**32**, 723-735  
42  
43  
44 885 Zuber, SM, Villamil, MB Meta-analysis approach to assess effect of tillage on microbial biomass and  
45 886 enzyme activities. *Soil Biology and Biochemistry*, 2016;**97**, 176-187  
46  
47  
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2 Are all families of bacteria capable of acquiring pathogenicity or are there barriers to the exchange of cer  
3 Are all functional traits horizontally transmissible?  
4 Are comparisons of microbial community composition at phylum level overused and uninformative?  
5 Are faunal and floral biodiversity driven by microbial communities?  
6 Are outdated species and subspecies definitions impeding our understanding of microbial ecosystems?  
7 Are prosperous and diverse microbial communities that can auto-regulate less harmful, or risk-prone, tha  
8 Are social scientists and economists going to be present in the panels on animal and plant health of the E  
9 Are species just a handful of relatively stable lineages within gene pools in which horizontal gene transfer  
10 Are studies which consider microbes by genus adequate in light of observed interspecies variation?  
11 Are synthetic communities useful in inferring theories about microbial community dynamics?  
12 Are the genes related with metabolic function vertically or horizontally inherited  
13 Are there any unexplored 'microbial frontiers'?  
14 Are there difference in microbiomes of heather under different management regimes?  
15 Are there differences between skin microbiome of rural compared to urban amphibians?  
16 Are there species or just highly integrated microbial communities?  
17 As climate change continues to impact the global ecology, can monitoring microbial communities give an  
18 As climate change effects soils including salinity, water levels and temperature, will this impact on food p  
19 As extreme environments are being perceived as offering the best option for novel antimicrobials what h  
20 As fracking looks increasingly likely to happen in the UK, what will be the effect on the surrounding micro  
21 As it is may already have gone past the tipping point for climate change, can evolution be monitored in ei  
22 At what spatial scale do we need to study microbial communities to answer applied or globally important  
23 Can a better understanding of microbial ecology give us clues on how complex larger ecosystems functio  
24 Can bacteriophages be used to eliminate certain bacteria in an environmental sample?  
25 Can bioinoculation based bioremediation techniques be deployed more successfully as a result of omics  
26 Can endohyphal bacteria positively impact the environment through their host?  
27 Can host community assemblages be used to predict parasite (micro and macro) community composition  
28 Can media monitoring be used for early identification of new emerging threats to animal and plant health  
29 Can metagenome analysis completely replace phylogenetic markers to describe diversity in microbiomes  
30 Can micro-organisms be cultivated directly in soils using greenhouses?  
31 Can microbes 'prime' each other to degrade organic matter?  
32 Can microbes go extinct?  
33 Can microbial communities ever be considered stable or is stability an artefact of the spatial scale by whi  
34 Can microbial ecology provide an alternative to antibiotics?  
35 Can microbial ecology tell us anything about non-microbial ecology?  
36 Can probiotic microbiota mitigate water-stress induced plant disease?  
37 Can regional outbreaks of exotic tree diseases become opportunities for nature conservation?  
38 Can the gut mirobione be manipulated to enhance health?  
39 Can understanding the relationship between ash die back disease and microbial community be used in sp  
40 Can we come up with a standardized method for estimating relative abundance of high-throughput data?  
41 Can we distinguish individual from interactive microbial effects? e.g. microbes that "turn on" (or off) pat  
42 Can we effectively synthesize microbial communities specialized in decomposing waste and recycling for  
43 Can we genetically engineer endohyphal bacteria that are known to be the cause of plant disease to exer  
44 Can we integrate our understanding of different microbiomes?

1  
2 Can we manipulate migratory bacteria in soil in a way that it's useful for applications other than bioremediation?  
3 Can we predict the functioning of communities from data on individual species within it (e.g. metabolic capabilities)?  
4 Can we quantify the role of microbes in the major global geochemical cycles (e.g. carbon, nitrogen, methane)?  
5 Can we study ancient microbial communities, and use that information to predict microbial community evolution?  
6 Do different functional clades have differing critical thresholds with regards to ecosystem disturbance?  
7 Do ecological interactions have a role in bacterial plasticity?  
8 Do metagenomics and amplicon sequencing provide useful information about the actual functionality of microbial communities?  
9 Do microbial communities have keystone species or an equivalent e.g. a keystone functional clade?  
10 Do microbial ecologists know enough microbiology?  
11 Do specific global ecosystems warrant particular focus and further study?  
12 Do the tenets of prokaryotic microbial ecology hold true for eukaryotic species and genera?  
13 Do we need models in microbial ecology?  
14 Do we see similar biogeographical patterns for microbes as for 'macrobes'? If not, why not?  
15 Does Applied Microbial Ecology exist?  
16 Does microbial ecology require more specialist lab equipment?  
17 Does the biodiversity of rhizosphere microorganisms change according to the type of root, depth of the root, or soil type?  
18 does the microbiota affect host behaviour?  
19 Does the presence of introduced fish in high mountain lakes impact on water quality and microbial communities?  
20 For a given microbial process, what spatial scale(s) does it operate at?  
21 Fracking releases the groundwater and may cause an increase in pollutants, how will this affect groundwater quality?  
22 Fungi can be found in various extreme environments. Why haven't we found many deep-sea fungi that are adapted to these conditions?  
23 Gaia theory - is there more to be explained by microbial ecology?  
24 How accurate are our measurements and understanding of in situ processes or are we extrapolating from laboratory data?  
25 How best can we harness insect microbial pathogens for biological control of crop pests?  
26 How best do we validate models of microbiomes through experiments and collecting samples from the field?  
27 How can a bacterial 'species' be defined?  
28 How can amplicon data be produced and analysed in a robust and comparable way?  
29 How can deep-sea fungi adapt to the lack of light and autotrophic organisms?  
30 How can microbial ecology inform conservation studies?  
31 How can microbial ecology best support the advancement of agriculture?  
32 How can microbiome studies improve personal medicine?  
33 How can systems approaches improve our understanding of host-microbe interactions?  
34 How can the economic and social relevance of microbial ecology be reinforced with funding and decision-making?  
35 How can we account for variations in genome length when measuring variations in abundance and diversity?  
36 How can we accurately measure soil microbial biomass in a reproducible manner?  
37 How can we advance our screening methods to map microbes from "extreme" environments?  
38 How can we apply modelling techniques to analyse the risks of ecological and agricultural probiotics?  
39 How can we assess the role of the unculturable microbiome in the ecology of real habitats?  
40 How can we better measure dispersal capacities of microbes?  
41 How can we better track particular microbes in communities?  
42 How can we compare the roles of decomposition between terrestrial and aquatic fungi?  
43 How can we culture communities of environmental microbes in situ?  
44 How can we develop suitable software programmes, tools, statistical approaches and databases to analyse microbial ecology data?



- 1
- 2 How can we examine ecological processes (e.g. N mineralisation) on a scale relevant to microbes?
- 3 How can we get young people interested in invisible, boring microbes?
- 4 How can we identify microbial species in the environment in real-time?
- 5
- 6 How can we improve the proportion of microbial diversity that is isolated and held in pure culture?
- 7 How can we interpret Koch's postulates in relation to polymicrobial infections as revealed by microbiome
- 8 How can we make microbial ecology relevant to policy makers?
- 9
- 10 How can we measure the hidden diversity? Overcoming arbitrary cut off values in next-generation sequ
- 11 How can we prove that microbes have ecosystem-scale impacts?
- 12 How can we relate large-scale taxonomic information to fine-scale function?
- 13
- 14 How can we solve the most pressing career issues affecting early stage researchers in microbial ecology?
- 15 How can we stop pathogens from forming biofilms?
- 16 How certain is genetic decay in a symbiont
- 17 How cosmopolitan are microorganisms?
- 18 How diverse is a 'community'?
- 19
- 20 How do differences in microbial communities in human and animal microbiomes relate to different disea
- 21 How do gut bacterial communities interact with viral infections? Can we classify communities as "neutral
- 22 How do microbial communities interact to effect the phenotype of the host?
- 23 How do plant endophytes enter, become active in, and influence their hosts?
- 24
- 25 How do protists influence fungi in soil?
- 26 How do skin microbiota and UV interact in amphibians, especially at high altitude?
- 27 How do soil bacteria, viruses and archaea interact?
- 28
- 29 How do virus influence microbial ecology?
- 30 How do we establish effective sample sizes for studies of poorly characterised microorganism?
- 31 How do we sample environmental variables at an appropriate scale that are proximate to microbial comr
- 32
- 33 How does individual microbiota affect others at a community level
- 34 How does the environment select?
- 35 How does the microbiome influence health?
- 36
- 37 How important is the "rare" microbiome in ecosystem function?
- 38 How important is the microbiota in mediating adaptation that results in species invasions?
- 39
- 40 How is best to study the changing resource environment of microbial communities?
- 41 How is climate change going to affect the microbial communities in the drinking water distribution syste
- 42 How much functional redundancy is there in microbes relative to higher taxa?
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- 44 How much functional redundancy is there in microbial communities, and how does that interact with divi
- 45 How much functional redundancy is there in the host microbiome?
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- 47 How much intra- vs inter-specific trait variation is there?
- 48 How much microbial diversity in the soil has been lost through monoculture and what is the importance (
- 49 How problematic is PCR amplification bias?
- 50 How similar are is the rhizosphere biodiversity of microbes from the same species of tree located in differ
- 51 How stable are microbial communities, and how can we assess their stability, particularly in changing env
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- 53 How to foster interdisciplinary approaches in and around microbial ecology?
- 54 How to solve the leaky pipeline of female microbial ecologists in academia?
- 55
- 56 How to solve the peer reviewer crisis in microbial ecology?
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- 58 How well do current species distribution models developed with microbial systems predict the distributic
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2 How will different temperature and humidity regimes reflecting likely climate change scenarios affect lea  
3 How will predicted climate changes influence the free-living stages of terrestrial and aquatic helminths?  
4 Intensive farming may involve high levels of broad spectrum antibiotic usage. This in turn will end up in t  
5 Is Alan McCarthy the oldest microbial ecologist on the planet?  
6 Is everything everywhere?  
7 Is it necessary/important to document microbial diversity? Should it be done at the genus/species level?  
8 Is it still relevant to dig for antibiotics?  
9 Is it syntrophy the main metabolic mechanism sustaining microbial cooperation  
10 Is it time to stop cataloguing bacterial community compositions in different habitats and put a bit more e  
11 Is microbial abundance and/or diversity driving ecosystem functioning?  
12 Is microbial ecology restricted by the red tape surrounding releasing microbes into the environment?  
13 Is the carbon source used in general/selective media the limiting factor for some un-culturable micro-org  
14 Is the growth in microbial ecology publication sustainable?  
15 Is there a relationship between microbial community and ecosystem functioning, and is it observed across  
16 Is there a way to overcome big data?  
17 Is there any point in doing comparative metagenomics?  
18 Is there going to be an improvement in the file-drawer problem (i.e. the tendency of positive results to be  
19 Is there such thing as a microbial pathogen?  
20 Is virus burden and shedding intensity in mammals amplified when co-infected by helminths?  
21 Many hands make light work? What is the role of functional redundancy in ecosystem processes?  
22 mechanisms extremophiles utilise for survival and potential uses elsewhere  
23 No biome is an island. As interest in this area increases, how do microbiomes interact outside the microsc  
24 Not Waving but Drowning; how much evidence do we need to fully interpret omics data?  
25 One size fits all? Is there a relationship between microbial and "macrobial" ecology?  
26 Pathogens get a lot of mainstream attention. Should researchers do more to promote positive microbial c  
27 plant host interactions, symbiotic relationships  
28 Rapid detection and identification of microbes  
29 Sequencing data has increased significantly in the last 15 years, will bioinformatics be the future of our st  
30 Should new species descriptions based entirely on sequences be allowed?  
31 Should researchers place greater emphasis on evaluating microbe functional traits (applied aspects), and  
32 Should we be concerned about the extinction of microbial species?  
33 Should we couple microbial ecology with Invasive Non-Native Species (INNS) risk assessments?  
34 Society and policy makers; do they value microbial ecosystem services?  
35 Soil microbial communities and the role they play  
36 Soil microbial communities and the role they play  
37 Soil profiling has been done for many years, can we estimate the type of organisms in a soil using only inf  
38 Taxonomy vs function: Do functional groups exist and in a world of horizontal gene transfer. Do 16S sequ  
39 The rare biosphere; waiting in the wings or ghosts in the machine?  
40 There are many factors that alter and select which organisms belong in a specific environment, througho  
41 To what extent are microbial species distributions influenced by climate? what would the consequences  
42 To what extent do microbial communities return to their "original" state after short-term perturbations?  
43 To what extent does microbial community composition reflect functional redundancy in a habitat or ecos  
44 To what extent is among human or animal variability in microbiome related to evolutionary processes occ

- 1  
2 To what extent is animal health and welfare influenced by their microbiome and does this have the poter  
3 Universal sequence primers - can they ever exist?  
4 What are the biggest gains in microbial ecology of the last decade and what are their implications for the  
5 What are the consequences of antibiotic use in microbial communities in freshwater and soil environmer  
6 What are the environmental hazards associated with bioremediation and biocontrol, and what can we dc  
7 What are the likely impacts of climate change on plant diseases in understudied regions such as Africa, th  
8 What are the links between microbial genomics and metabolomics?  
9 What are the main biotic and abiotic determinants of population structure in microbial communities in w  
10 What are the main mechanisms by which nutrients accumulated by saprotrophs are released to the envir  
11 What are the major constraints on microbial life on land and water, and how do they differ?  
12 What are the mechanisms by which the gut microbiota protects its host from pathogens?  
13 What are the mechanisms used by mycoviruses to confer host fitness to its environment?  
14 What are the microbial ecology consequences of the cultivation of exotic tree species?  
15 What are the most successful applications of microbial ecology in the real world?  
16 What are the opportunities to determine cause or effect relationships in microbiome studies?  
17 What are the primary mechanisms that mediate microbial interactions within a host? and the strength of  
18 What are the survival implications of skin and gut microbiomes of captive bred or head-started animals?  
19 What are the traits of microbial species that are a) globally ubiquitous b) endemic to certain areas?  
20 What can animal, human and plant health policy-makers learn from evolutionary ecology?  
21 What effect will rising water levels have on coastal microbes and their diversity?  
22 What is the 'functioning' of a microbial community?  
23 What is the acceptable amount of disease in a healthy forest ecosystem?  
24 What is the actual relevance of Black Queen Hypothesis  
25 What is the average carbon footprint of a paper in microbial ecology and how can it be reduced?  
26 What is the best method to assess the contribution of rare vs abundant species in a microbial community  
27 What is the best strategy to control and stop waterborne outbreaks?  
28 What is the best way of monitoring drinking water safety?  
29 What is the ecological meaning of an OTU?  
30 What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms of thei  
31 What is the importance of biotic vs abiotic characteristics in determining microbial community compositi  
32 What is the importance of photosynthetic symbionts of larval amphibians?  
33 What is the relative importance of inter- and intra-specific diversity in fungal communities?  
34 What is the relative importance of stochastic vs. determinative processes in fungal community assembly?  
35 What is the relative role of competition versus cooperation for microbial biodiversity  
36 What is the role of bacteriophages in biofilm dynamics in natural environments?  
37 What is the role of the microbiota in speciation processes?  
38 What is the true link between diversity and function in an ecosystem?  
39 What mechanisms are distinct in microbial ecology as opposed to conventional ecology?  
40 What methodological processes do we need to develop to give a holistic view of microbial diversity and f  
41 What methods can we use to marry microbial diversity with function, what role could proteomics play?  
42 What new sequencing techniques do we need to improve our understanding of microbial ecology?  
43 What new technologies are available to detect cross-feeders in an environmental sample?  
44 What proportion of fungi carry endobacteria, and what effect does this have?  
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2 What scales are appropriate for studying microbes?  
3 What selection pressures does the modern world place on evolving microbes?  
4 What technologies are needed to advance microbial ecology?  
5 What's the best way to manipulate the microflora?  
6 Which factors determine the host range of microbial pathogens?  
7 Which factors determine the structure of gut microbiomes?  
8 Which factors determine whether Wolbachia increases or decreases its host's susceptibility to pathogens  
9 Which factors trigger 'covert' infections to become 'overt', impacting host health?  
10 Which factors trigger the emergence of new microbial pathogens in humans and wildlife?  
11 Which is the most effective way of disinfecting drinking water?  
12 Why are some microbial pathogens often 'covert'?  
13 Why do genomes contain redundant copies of genes  
14 Will 16S rRNA be supplanted as the key phylogenetic identifier  
15 Will fungal underrepresentation in the life sciences keep on decreasing as was the case over the last two  
16 Will metagenomics ever supplant next generation sequencing?  
17 Will metaproteomics ever become an important major methodology in microbial ecology?  
18 Will microbes become commonly used as biological control agents of invasive alien plants in Europe?  
19 Will ocean acidification or temperature increase provide the greatest selective pressure for microbial ma  
20 Will the challenge of fungal genomics to the dogma of name-based biosecurity be taken on board by phy  
21 Will the culturing of the other 99% of bacteria be possible with increasing understanding of syntrophy an  
22 Will the flood of new papers on climate change and microbial ecology lead to increased specialization an  
23 Will the use of network theory in plant epidemiology help prevent plant health emergencies such as Euro  
24 Will there be substantial funding for research and management of tree diseases over the next decades, o  
25 Will there ever be a cohesive species concept which applies to bacteria?  
26 Will there ever be a unified V 16S rRNA region set of primers to capture total diversity  
27 Will we be able to find new antibiotics in microbial species? Should we prioritise funding for this?  
28 Will we ever be able to differentially monitor infective and non infective viruses in the environment?  
29 With specific reference to non-model organisms. We know metazoa harbour a diverse array of microbial  
30 With the amount of -omics sequence information coming out every week, how can scientists is it worth t  
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tain genetic elements which are required?

an disinfected zones whereby resistant microbes have the potential to thrive and be pathogenic?  
European Food Safety Authority?  
is rife?

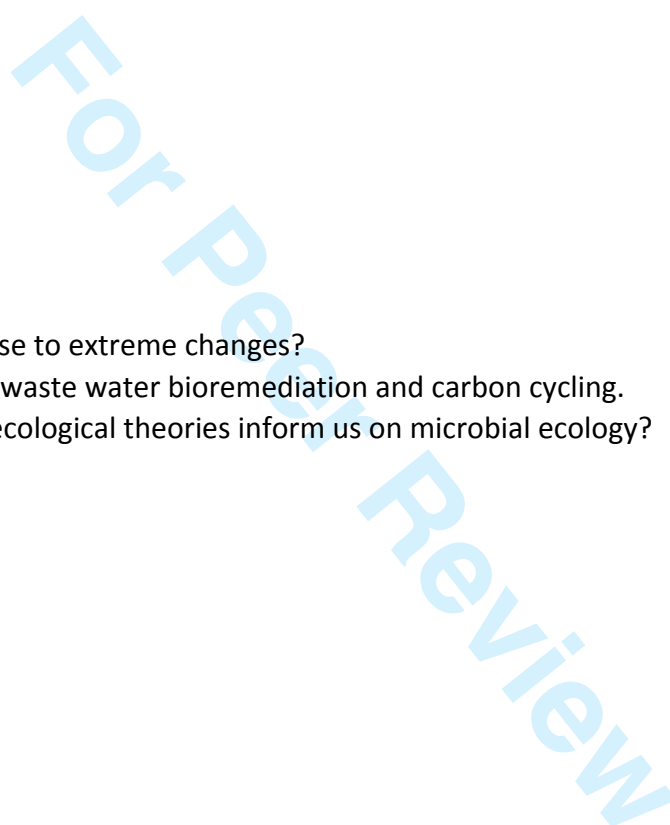
insight into adaptability?  
roduction?  
appens next?  
brial population?  
merging species in response to extreme changes?  
t questions? For example, waste water bioremediation and carbon cycling.  
n? Inversely, can current ecological theories inform us on microbial ecology?

i based techniques?

o?  
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ch the community is investigated?

pecies conservation?  
? And will there be a way to measure absolute abundance using current sequencing methods?  
rogenicity in other microbes?  
industrial purposes?  
t positive effects instead?



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3 capacities, genomes)?  
4 (ane, etc)? What are the implications of microbial species loss in these cycles?  
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6 evolution?  
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10 microbial communities or do this methods get bias from collecting too much material from dead  
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22 root, size of the whole tree, maturity of the tree?  
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25 nunity in streams?  
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27 r microbiota?  
28 re presumably barophilic?  
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31 n experimental artifacts?  
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44 making bodies  
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46 sity in soil microbial ecosystems?  
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58 se ~big data~ projects?  
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studies?

encing.

se conditions?  
ising" and "enhancing"?

unities which allows us to progress our understanding of what is structuring these communities

ns and therefore drinking water safety?

ersity and niche overlap?

of this?

rent places? What are the factors that affect this community most significantly?  
vironments (e.g. seasonal, tidal, subject to frequent disturbance)?

ons of microbes?could new software improve on these limitations?

For Peer Review



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2 f microbiota of umbrella species?  
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4 he faecal matter of livestock and deposit on the land. What will be the long term effect on micro  
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12 effort into virus and microeukaryote ecology?  
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16 organisms?  
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19 is ecosystems (e.g. marine, freshwater, terrestrial, gut microbiota)?  
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23 e more easily published than negative ones) in microbial ecology?  
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30 biome environment?  
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34 effects?  
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38 studies of microbial ecology?  
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41 less on documenting diversity?  
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48 information about the abiotic factors of a soil?  
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50 ences mean anything?  
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53 ut the years we have established the importance of temperature, pH, moisture, basic factorsâ€¦  
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55 of any resulting microbiome shift for agriculture and biodiversity?  
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58 system?  
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60 ccurring within an individual's microbiome?

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potential to affect conservation efforts?

future of the field?

risks and how they influence human health?

ways to prevent them?

in the Middle East and Central America?

water distribution systems?

environment?

is there evidence for them?

is there?

their survival and spread?

on, and how does that influence ecosystem functioning?

is there?

function?

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19 decades?

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24 rine organisms?

25 tosanitary regulators?

26  
27 d the rise of metabolomic and transcriptomic studies

28 d communication problems within the field?

29 epan ash dieback?

30 r is the recent surge in the UK a short-lived burst?

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38 diversity. How can changes in microbial communities, either through changes in community com  
39 he while for scientists to create alliances so this information can be quickly available for other sci  
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5 microbial population as a driver for antibiotic resistance?  
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53 which are the new most important factors that are of interest to microbial ecologists?  
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position or microbial evolution, help long lived (annual +) animals adapt to rapidly changing environments that might be interested in the same information? Joining forces throughout societies.

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For Peer Review