


Please cite the Published Version

Antwis, RE, Griffiths, SM , Harrison, XA, Aranega-Bou, P, Arce, A, Bettridge, AS, Brailsford, FL, de Menezes, A, Devaynes, A, Forbes, KM, Fry, EL, Goodhead, I, Haskell, E, Heys, C, James, C, Johnston, SR, Lewis, GR, Lewis, Z, Macey, MC, McCarthy, A, McDonald, JE, Mejia-Florez, NL, O'Brien, D, Orland, C, Pautasso, M, Reid, WDK, Robinson, HA, Wilson, K and Sutherland, WJ (2017) Fifty important research questions in microbial ecology. FEMS Microbiology Ecology, 93 (5). ISSN 0168-6496

DOI: <https://doi.org/10.1093/femsec/fix044>

Publisher: Oxford University Press (OUP)

Version: Accepted Version

Downloaded from: <https://e-space.mmu.ac.uk/621865/>

Usage rights:  In Copyright

Additional Information: This is an Author Accepted Manuscript of a paper accepted for publication in FEMS Microbiology Ecology, published by and copyright Oxford University Press (OUP).

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

<http://mc.manuscriptcentral.com/fems>

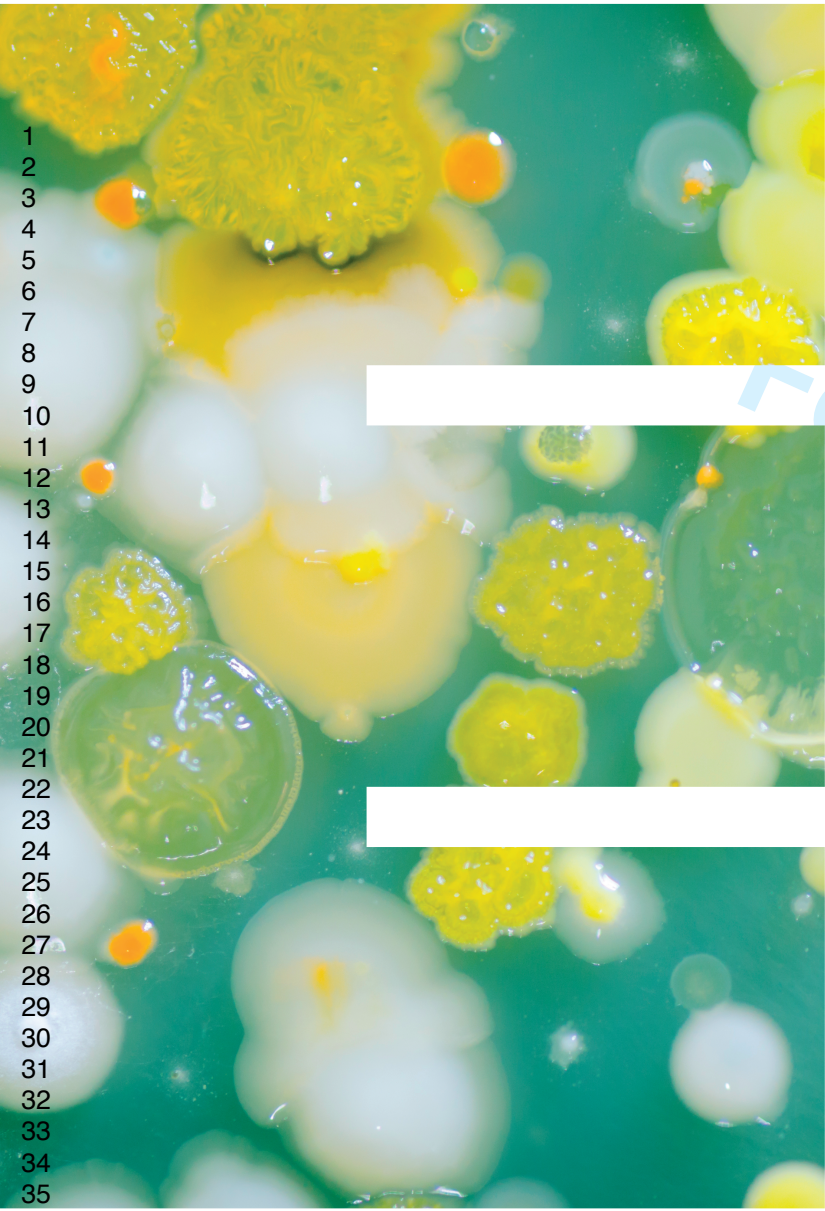
50 important research questions in microbial ecology

Journal:	<i>FEMS Microbiology Ecology</i>
Manuscript ID	FEMSEC-16-11-0599.R2
Manuscript Type:	Perspective
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Antwis, Rachael; University of Salford, School of Environment and Life Sciences</p> <p>Griffiths, Sarah; Manchester Metropolitan University, School of Science and the Environment</p> <p>Harrison, Xavier; Zoological Society of London, Institute of Zoology</p> <p>Aranega-Bou, Paz; University of Salford, School of Environment and Life Sciences</p> <p>Arce, Andres; Imperial College London, Faculty of Natural Sciences</p> <p>Bettridge, Aimee; Cardiff University, School of Biosciences</p> <p>Brailsford, Francesca; Bangor University, School of Environment, Natural Resources and Geography</p> <p>de Menezes, Alexandre ; University of Salford, School of Environment and Life Sciences</p> <p>Devaynes, Andrew; Edge Hill University, Biosciences</p> <p>Forbes, Kristian; University of Helsinki, Department of Virology</p> <p>Fry, Ellen; University of Manchester, Faculty of Life Sciences</p> <p>Goodhead, Ian; University of Salford, School of Environment and Life Sciences</p> <p>Haskell, Erin; University of York, Department of Biology</p> <p>Heys, Chloe; University of Liverpool, Institute of Integrative Biology/School of Life Sciences</p> <p>James, Chloe; University of Salford, School of Environment and Life Sciences</p> <p>Johnston, Sarah; Cardiff University, Cardiff School of Biosciences</p> <p>Lewis, Gillian; Edge Hill University, Biosciences</p> <p>Lewis, Zenobia; University of Liverpool, Institute of Integrative Biology/School of Life Sciences</p> <p>Macey, Michael; University of East Anglia, School of Environmental Sciences</p> <p>McCarthy, Alan; University of Liverpool, Microbiology Research Group</p> <p>McDonald, James; Bangor University College of Natural Sciences, School of Biological Sciences</p> <p>Mejia Florez, Nasmille ; University of East Anglia, School of Environmental Sciences</p> <p>O'Brien, David; Scottish Natural Heritage, Scottish Natural Heritage</p> <p>Orland, Chloé; University of Cambridge, Department of Plant Sciences</p> <p>Pautasso, Marco; European Food Safety Authority, Animal and Plant Health Unit</p>

	Reid, William; Newcastle University, School of Biology Robinson, Heather; University of Manchester, Faculty of Life Sciences Wilson, Ken; Lancaster University, Lancaster Environment Centre Sutherland, William; University of Cambridge, Department of Zoology
Keywords:	environmental processes, evolutionary processes, functional diversity, host-microbiome interactions, priority setting, research agenda

SCHOLARONE™
Manuscripts

This is a pre-copyedited, author-produced version of an article accepted for publication in FEMS Microbiology Ecology following peer review. The version of record Antwis et al. (2017) is available online via: <https://doi.org/10.1093/femsec/fix044>



MICROBIAL

ECOLOGY

GROUP

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

1
2
3 **1 50 important research questions in microbial ecology**
4
5
6
7

8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2
3 Rachael E. Antwis^{*1}, Sarah M. Griffiths², Xavier A. Harrison³, Paz Aranega-Bou¹, Andres Arce⁴, Aimee
4 S. Bettridge⁵, Francesca L. Brailsford⁶, Alexandre de Menezes¹, Andrew Devaynes⁷, Kristian M.
5 Forbes⁸, Ellen L. Fry⁹, Ian Goodhead¹, Erin Haskell¹⁰, Chloe Heys¹¹, Chloe James¹, Sarah R.
6 Johnston⁵, Gillian R. Lewis⁷, Zenobia Lewis¹¹, Michael C. Macey¹², Alan McCarthy¹¹, James E.
7 McDonald¹³, Nasmille L. Mejia Florez¹², David O'Brien¹⁴, Chloé Orland¹⁵, Marco Pautasso¹⁶, William
8 D. K. Reid¹⁷, Heather A. Robinson⁹, Kenneth Wilson¹⁸, William J. Sutherland¹⁹
9

10 *Corresponding author:

11 Rachael E. Antwis, Room 336, Peel Building, University of Salford, The Crescent, Salford, M5 4WT,
12 UK; r.e.antwis@salford.ac.uk
13

14 Affiliations:

- 15 1. School of Environment and Life Sciences, University of Salford, Salford, UK
- 16 2. School of Science and the Environment, Manchester Metropolitan University, Manchester, UK
- 17 3. Institute of Zoology, Zoological Society for London, London, UK
- 18 4. Silwood Park, Faculty of Natural Sciences, Imperial College London, UK
- 19 5. School of Biosciences, Cardiff University, Cardiff, UK
- 20 6. School of Environment, Natural Resources and Geography, Bangor University, Bangor, UK
- 21 7. Biosciences, Edge Hill University, Ormskirk, UK
- 22 8. Department of Virology, University of Helsinki, Helsinki, Finland
- 23 9. School of Earth and Environmental Sciences, Faculty of Science and Engineering, University of
24 Manchester, Manchester, UK

25

- 1
2
3 26 10. Department of Biology, University of York, York, UK
4
5 27 11. Institute of Integrative Biology/School of Life Sciences, University of Liverpool, Liverpool, UK
6
7 28 12. School of Environmental Sciences, University of East Anglia, Norwich, UK
8
9
10 29 13. School of Biological Sciences, Bangor University, Bangor, UK
11
12 30 14. Scottish Natural Heritage, Inverness, UK
13
14 31 15. Department of Plant Sciences, University of Cambridge, Cambridge, UK
15
16
17 32 16. Animal and Plant Health Unit, European Food Safety Authority, Parma, Italy
18
19 33 17. School of Biology, Newcastle University, Newcastle upon Tyne, UK
20
21 34 18. Lancaster Environment Centre, Lancaster University, Lancaster, UK
22
23 35 19. Conservation Science Group, Department of Zoology, University of Cambridge, Cambridge, UK
24
25

26 36

27 37

28 38 Keywords:

29 39 environmental processes, evolutionary processes, functional diversity, host-microbiome interactions,

30 40 priority setting, research agenda
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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).

80

81

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

29 100

30
31 101 A total of 244 questions were submitted by attendees (see Supplementary Information), and assigned
32
33 102 (by R.E. Antwis and S.M. Griffiths) to the following themes;
34

- 35 103 1) *Host-Microbiome Interactions*;
36
37 104 2) *Health and Infectious Diseases*;
38
39 105 3) *Human Health and Food Security*;
40
41 106 4) *Microbial Ecology in a Changing World*;
42
43 107 5) *Environmental Processes*;
44
45 108 6) *Functional Diversity*;
46
47 109 7) *Evolutionary Processes*.
48
49
50

51 110

52
53
54 111 An additional eighth theme named '*Society and Policy*' was created to capture a number of questions
55
56 112 that were generally applicable across the biological sciences, as well as a number of questions
57
58
59
60

1
2
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*.

6
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
28
29 126 duplicates removed and questions reworded as necessary. For each theme, a final set of 'gold' (~15%
30
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
38
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
42
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.

46
47 135

48
49 136 *Limitations*

50
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
54
55 139 a physical workshop and via the British Ecological Society) means that, without a substantial travel
56
57 140 budget, a bias towards UK institutions was inevitable. However, many participants have worked on, or
58
59
60

1
2
3 141 currently collaborate in, research projects on non-UK ecosystems and species, and therefore the
4
5 142 questions proposed are drawn from considerable knowledge and experience of the field
6
7 143 internationally. Additionally, although most individuals were from academic institutions, many
8
9 144 individuals had previous or on-going collaborations with industrial partners and governmental/non-
10
11 145 governmental organisations.

12
13 146

14 147 **Results**

15
16
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
20
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
28
29 154 temporal and spatial scales.

30
31 155

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

34
35 157 Host-microbiome interactions determine many host life history traits such as behaviour, reproduction,
36
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,
60

1
2
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.
6

7 172

8
9 173 1. What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe
10
11 174 interactions?

12
13 175 2. What are the relative contributions of host-associated and environmental factors in determining host
14
15 176 microbial community composition?

16
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?

22
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
33 185

34
35
36
37 186 ***Health and Infectious Diseases***

38
39 187 The last 50 years have seen the emergence of several hypervirulent wildlife pathogens in animals
40
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.*,
44
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 &
48
49 192 Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.*, 2016). A major outstanding research goal is
50
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
54
55 195 studies have sought to determine how manipulation of host microbiomes may ameliorate the spread
56
57 196 and impact of such diseases (e.g. Rebollar *et al.*, 2016).
58
59
60

1
2
3 197 While for many disease states the paradigm holds true that one microorganism causes one
4
5 198 disease, polymicrobial infections are becoming more apparent through metagenomic and
6
7 199 metatranscriptomic sequencing of disease-associated microbial communities (Gilbert *et al.*, 2016).
8
9 200 Consequently, the 'pathobiome' concept, where a disease state is influenced by complex interactions
10
11 201 between commensal and pathogenic microorganisms, presents new challenges for applying Koch's
12
13 202 postulates to diseases arising from polymicrobial interactions (Vayssier-Taussat *et al.*, 2014), such as
14
15 203 black band disease (BBD) in corals (Sato *et al.*, 2016) and olive knot disease (Buonaurio *et al.*, 2015).

16
17 204 In this theme we have identified research questions relating to the microbial ecology of
18
19 205 infectious diseases and host health. Although much can be learnt from the comparatively high number
20
21 206 of studies in the human and biomedical literature (*e.g.* using network approaches in epidemiology), the
22
23 207 questions selected in this theme predominantly relate to non-human animals and plants, as humans
24
25 208 are covered later (*'Human Health and Food Security'*).

26
27 209

28
29 210 9. How can we better track the source and dispersal of particular microorganisms in real time?

30
31 211 10. Many microorganisms are unculturable, and many microbiome studies reveal that diseases are
32
33 212 polymicrobial; how can we re-evaluate Koch's postulates in this context?

34
35 213 11. Which factors trigger 'covert' infections to become 'overt', impacting host health?

36
37 214 12. At the population level, how is the burden and shedding intensity of intracellular microbes affected
38
39 215 by co-infection by extracellular parasites?

40
41 216 13. What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms
42
43 217 of their survival and spread?

44
45 218 14. How can network theory best be used to predict and manage infectious disease outbreaks in
46
47 219 animals and plants?

48
49 220 15. Can microbiomes of wildlife (plants and animals) be used or manipulated to enhance health and/or
50
51 221 disease resistance?

52
53 222

54
55
56 223 ***Human Health and Food Security***
57
58
59
60

1
2
3 224 With the human population due to exceed eight billion by 2024, food security and human health are
4
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-
28
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-
34
35 240 intensity monoculture crops have a negative impact on the microbial biodiversity of soils through a
36
37 241 combination of tillage, subsequent erosion and chemical applications (Helgason *et al.*, 1998; Jacobsen
38
39 242 and Hjelmsø, 2014; Zuber and Villamil, 2016), which imposes selection pressures on pathogenic
40
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.

50
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.
60

- 1
2
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?
14
15 260 20. To what extent are microbial species distributions influenced by climate, and what are the
16
17 261 consequences for food security and human health?
18
19 262 21. How much microbial diversity in the soil has been lost through monoculture and what is the
20
21 263 importance of this?
22
23 264 22. Intensive farming may involve high levels of agrochemicals and broad-spectrum antibiotic usage -
24
25 265 what will be the long-term effects on microbial communities?
26
27 266 23. How best can we harness microbial communities to enhance food production?
28
29
30
31

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

34
35 269 Global changes resulting from human activity impact almost every habitat on earth. It is imperative that
36
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
60

1
2
3 282 such as CO₂, 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?

43
44
45 303

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

49
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

1
2
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
55
56 336 ecology (Widder *et al.*, 2016). This is true across a vast range of spatial scales, from microbial dyads
57
58 337 to the gut of a *Drosophila* fly, to ancient trees and their associated ecosystems, right through to global

1
2
3 338 biogeochemical processes. There is an urgent need to understand how the genome of a microbial
4 339 community (and in some cases, its host) relates to metabolic capacities. Conversely, there is also a
5 340 need to understand how ecosystems depend on a particular organism or group of organisms for any
6 341 given process and function. This section describes the need to move from simply describing microbial
7 342 diversity to understanding what these organisms are doing, how they are doing it, and what biotic and
8 343 abiotic drivers are controlling their activity. Each question may derive a suite of different answers,
9 344 depending on the group of organisms, the habitat and the process.

10
11
12
13
14
15
16 345

17
18 346 36. What are the mechanisms driving microbial community structure and function, and are these
19 347 conserved across ecosystems?

20
21
22 348 37. What is the relative importance of stochastic vs. determinative processes in microbial community
23 349 assembly?

24
25
26 350 38. How conserved are microbial functions across different spatial and temporal scales?

27
28
29 351 39. What is the relative importance of individual 'species' for the functioning of microbial communities?

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

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

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

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

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

50
51
52 362

53
54
55 363 ***Evolutionary Processes***

56
57 364 The role of microorganisms in determining evolutionary outcomes of hosts is being investigated in
58
59
60

1
2
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?

48
49 389 **Society and Policy**

50
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
57
58
59
60

1
2
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.
7
8

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?
26
27

28 405

30 406 **Discussion**

31
32
33 407 Here we present 50 important research questions across a number of themes relating to the field of
34
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
38
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
57
58 420 research areas, for example the application of information from human microbiome studies to the
59
60

1
2
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

1
2
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
59
60 480 well as for methods and protocols, was reflected in the questions shortlisted for the '*Society and*

1
2
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.

19
20
21
22
23
24
25
26
27
28
29
30
31
32
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

1
2
3 511 expansion in methodological approaches that have improved our understanding of microbial
4
5 512 community diversity and function. That said, other areas of microbial ecology should not be ignored or
6
7 513 forgotten. Given the rapidly evolving field of microbial ecology, it is expected that future workshops
8
9 514 with a wide draw will be held to ensure that the identification of research priorities and areas of interest
10
11 515 is a continuing process.

12
13 516

14 15 517 **Funding**

16
17 518 This work was supported by contributions from the British Ecological Society and the University of
18
19 519 Salford towards funding the workshop. SRJ is funded by NERC studentship NE/L501773/1, KMF by
20
21 520 the Finnish Cultural Foundation, NLMF is funded by Colciencias, MCM by Earth and Life Systems
22
23 521 Alliance, and WJS by Arcadia. The positions and opinions presented in this article are those of the
24
25 522 authors alone and are not intended to represent the views or scientific works of the European Food
26
27 523 Safety Authority.

28
29 524

30 31 525 **Acknowledgements**

32
33 526 Our thanks go to Francis Brearley and Michael Cunliffe for their submissions to the pre-workshop
34
35 527 questions, and to Jessica Hall, Daniel Henk and Matt Lloyd Jones for their contributions to the
36
37 528 workshop.

38
39 529

40 41 530 **References**

42
43
44 531 Allison, S, Martiny, J Resistance, resilience, and redundancy in microbial communities. *PNAS*,
45
46 532 2008;**105 S1**, 11512-11519.
47
48 533 Anderson, TH Microbial eco-physiological indicators to asses soil quality. *Agric Ecosyst Environ*,
49
50 534 2003;**98**, 285–293
51
52 535 Archie, EA, Theis, KR Animal behaviour meets microbial ecology. *Anim Behav*, 2011;**82**, 425-436
53
54
55 536 Baothman, OA, Zamzami, MA, Taher, I *et al.* The role of Gut Microbiota in the development of obesity
56
57 537 and Diabetes. *Lipids Health Dis*, 2016;**15**, 108.
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
4 565 catabolism, and their importance in nutrient cycling. *Current Opinion in Chemical Biology*, 2016;**31**, 50-
5 566 57
6
7
8 567 Chisholm, ST, Coaker, G, Day, B, *et al.* Host-microbe interactions: Shaping the evolution of plant
9 568 immune response. *Cell* 2006;**126**, 803-814
10
11 569 Cho, I, Blaser, MJ. The human microbiome: at the interface of health and disease. *National Review of*
12 570 *Genetics*, 2013;**13**, 260-270
13
14
15 571 Coker, JA Extremophiles and biology: current uses and prospects. *F1000 Research*, 2016;**5**, F1000
16 572 FacultyRev-396
17
18 573 Creamer, CA, de Menezes, AB, Krull, ES, *et al.* Microbial community structure mediates response of
19 574 soil C decomposition to litter addition and warming. *Soil Biol Biochem*, 2015;**80**, 175-188
20
21 575 Daskin, JH, Alford, RA Context-dependent symbioses and their potential roles in wildlife diseases.
22 576 *Proc Bio Sci.* 2012;**279**, 1457-65
23
24
25 577 Delgado-Baquerizo, M, Giaramida, L, Reich, P, *et al.* Lack of functional redundancy in the relationship
26 578 between microbial diversity and ecosystem functioning. *J. Ecol*, 2016;**104**, 936–946
27
28
29 579 Dicks, LV, Abrahams, A, Atkinson, J, *et al.* Identifying key knowledge needs for evidence-based
30 580 conservation of wild insect pollinators: a collaborative cross-sectoral exercise. *Insect Conservation*
31 581 *and Diversity*, 2012;**6**, 435-446
32
33
34 582 Dicks, LV, Bardgett, RD, Bell, J, *et al.* What do we need to know to enhance the environmental
35 583 sustainability of agriculture? A prioritisation of knowledge needs for the UK food system. *Sustainability*,
36 584 2013;**5**, 3095–3115
37
38
39 585 Dumont, MG, Murrell, JC. Community-level analysis: key genes of aerobic methane oxidation.
40 586 *Methods Enzymol*, 2015;**397**, 413-427.
41
42
43 587 El Khawand, M, Crombie, AT, Johnston, A, *et al.* Isolation of isoprene degrading bacteria from soils,
44 588 development of isoA gene probes and identification of the active isoprene degrading soil community
45 589 using DNA-stable isotope probing. *Environ Microbiol*, 2016;**18**, 2743-53
46
47
48
49
50
51
52
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
5 591 and Their Potential for Management. *BioMed Research International*, 2014;531824
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 [ScholarOne Support 1-434/964-4100](http://www.euro.who.int/en/publications/abstracts/tackling-antibiotic-resistance-from-a-food-safety-
39 742 <u>perspective-in-europe</u> [accessed 19 July 2016].
40
41
42
43
44 743 Widder, S, Widder, S, Allen, RJ <i>et al.</i> Challenges in microbial ecology: building predictive
45 744 understanding of community function and dynamics. <i>ISME J</i>, 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. <i>Nat Clim Change</i>, 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. <i>Nat Rev Microbio</i>, 2011;9, 233-243
54
55
56
57
58
59
60</p></div><div data-bbox=)

- 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
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7 **1 50 important research questions in microbial ecology**

8
9 2
10
11 3 Rachael E. Antwis^{*1}, Sarah M. Griffiths², Xavier A. Harrison³, Paz Aranega-Bou¹, Andres Arce⁴, Aimee
12 4 S. Bettridge⁵, Francesca L. Brailsford⁶, Alexandre de Menezes¹, Andrew Devaynes⁷, Kristian M.
13 5 Forbes⁸, Ellen L. Fry⁹, Ian Goodhead¹, Erin Haskell¹⁰, Chloe Heys¹¹, Chloe James¹, Sarah R.
14 6 Johnston⁵, Gillian R. Lewis⁷, Zenobia Lewis¹¹, Michael C. Macey¹², Alan McCarthy¹¹, James E.
15 7 McDonald¹³, Nasmille L. Mejia Florez¹², David O'Brien¹⁴, Chloé Orland¹⁵, Marco Pautasso¹⁶, William
16 8 D. K. Reid¹⁷, Heather A. Robinson⁹, Kenneth Wilson¹⁸, William J. Sutherland¹⁹

17
18
19
20
21 9
22
23 10 *Corresponding author:

24
25 11 Rachael E. Antwis, Room 336, Peel Building, University of Salford, The Crescent, Salford, M5 4WT,
26 12 UK; r.e.antwis@salford.ac.uk

27
28
29 13
30
31 14 Affiliations:

- 32
33 15 1. School of Environment and Life Sciences, University of Salford, Salford, UK
34
35 16 2. School of Science and the Environment, Manchester Metropolitan University, Manchester, UK
36
37 17 3. Institute of Zoology, Zoological Society for London, London, UK
38
39 18 4. Silwood Park, Faculty of Natural Sciences, Imperial College London, UK
40
41 19 5. School of Biosciences, Cardiff University, Cardiff, UK
42
43 20 6. School of Environment, Natural Resources and Geography, Bangor University, Bangor, UK
44
45 21 7. Biosciences, Edge Hill University, Ormskirk, UK
46
47 22 8. Department of Virology, University of Helsinki, Helsinki, Finland
48
49 23 9. School of Earth and Environmental Sciences, Faculty of Science and Engineering, University of
50 24 Manchester, Manchester, UK

51
52
53 25
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7 26 10. Department of Biology, University of York, York, UK
8
9 27 11. Institute of Integrative Biology/School of Life Sciences, University of Liverpool, Liverpool, UK
10
11 28 12. School of Environmental Sciences, University of East Anglia, Norwich, UK
12
13 29 13. School of Biological Sciences, Bangor University, Bangor, UK
14
15 30 14. Scottish Natural Heritage, Inverness, UK
16
17 31 15. Department of Plant Sciences, University of Cambridge, Cambridge, UK
18
19 32 16. Animal and Plant Health Unit, European Food Safety Authority, Parma, Italy
20
21 33 17. School of Biology, Newcastle University, Newcastle upon Tyne, UK
22
23 34 18. Lancaster Environment Centre, Lancaster University, Lancaster, UK
24
25 35 19. Conservation Science Group, Department of Zoology, University of Cambridge, Cambridge, UK
26
27 36
28
29 37
30
31 38 Keywords:
32
33 39 environmental processes, evolutionary processes, functional diversity, host-microbiome interactions,
34 40 ~~methods~~, priority setting, research agenda
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4
5
6
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.

10 91

11 92 *Questions*

12 93 Prior to the workshop, attendees were asked to submit questions via an online form that they thought

13 94 most closely met the following brief:

14 95 "We are aiming to identify 50 questions that, if answered, will make a considerable

15 96 difference to the use of microbial ecology by practitioners and policy makers, or to

16 97 the fundamentals of the field of microbial ecology. These should be questions that

17 98 are unanswered, could be answered, and could be tackled by a research

18 99 programme. This is expected to set the agenda for future research in the field of

19 100 microbial ecology."

20 101

21 102 A total of 244 questions were submitted by attendees (see Supplementary Information), and assigned

22 103 (by R.E. Antwis and S.M. Griffiths) to the following ~~eight~~ themes;

23 104 1) *Host-Microbiome Interactions*;

24 105 2) *Health and Infectious Diseases*;

25 106 3) ~~*Food Security and Human Health*~~ *and Food Security*;

26 107 4) *Microbial Ecology in a Changing World*;

27 108 5) *Environmental Processes*;

28 109 6) *Functional Diversity*;

29 110 7) *Evolutionary Processes*;

30 111 ~~8) *Methods in Microbial Ecology*.~~

31 112

32 113 An additional ~~ninth~~ ~~eighth~~ theme named '*Society and Policy*' was created to capture a number of

1
2
3
4
5
6
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
9
10 116 laboratory based microbial ecology research, *per se*.

11 117

12
13
14 118 *Question selection process*

15
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~~.

35 137

36
37
38 138 *Limitations*

39 139 All but four participants were from British universities, although there were representatives from a
40 140 range of nationalities and research areas. The manner in which this paper was developed (*i.e.* through
41 141 a physical workshop and via the British Ecological Society) means that, without a substantial travel

1
2
3
4
5
6
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
9
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-~~
44 164 ~~Rosenberg & Rosenberg, 2008; Daskin & Alford, 2012).~~ Increasingly, we are discovering that host-
45
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
55
56 170 likely important for global issues, such as the efficacy of conservation efforts including species

1
2
3
4
5
6
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.

16 177
17
18 178
19
20 179 1.4. What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe
21
22 180 interactions?

Formatted: Font: (Default) Arial, 10 pt,
Font color: Text 1

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

Formatted: Font: (Default) Arial, 10 pt,
Font color: Text 1

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?

40 192

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.*,

1
2
3
4
5
6
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 &
9
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*).

39
40 218
41
42 219 [948. How can we better track the source and dispersal of particular microorganisms in real time?](#)

43
44 220
45
46 221
47
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?

51
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

Formatted: Level 1, Indent: First line: 0"

1
2
3
4
5
6
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-
9
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-
14
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.*,~~
23
24 265 ~~2014). As antibiotic resistance increases, along with our concern about potential impact on both~~
25
26 266 ~~human and animal health, there is an increasing drive to find new forms of antibiotics.~~

27
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~~
32 270 ~~development of personalized medicine and novel antibiotics; and ii) understanding how current~~
33 271 ~~antibiotic regimes and farming practices may negatively impact the diversity of the environmental~~
34 272 ~~microbiome and food production capacity. This section examines ways in which we can harness~~
35 273 ~~microbial functions to improve overall human health through managing gut microbiota, and improve~~
36 274 ~~the soil and plant microbiome, thus increasing yields and associated biodiversity.~~

37
38 275
39
40 276 163. How can human microbiome studies improve personalised medicine?

41
42 277 174. What ecological principles can be applied in the search for new antibiotics and alternatives?

43
44 278 185. What are the main determinants of waterborne infection outbreaks, and what is the best strategy
45 279 to control these in water distribution systems?

46
47 280 196. What are the consequences of antibiotic and pharmaceutical use in human medicine on microbial
48 281 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 CO₂, 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

1
2
3
4
5
6
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.
17
18
19
20
21 320

22 321 240. How can we integrate microbial communities into models of global change?

23 322 254. Will ocean acidification, temperature increases and rising sea levels lead to changes in microbial
24 323 diversity or function, and what will the cascading effects of this be?

25 324 262. How do human activities, such as oil and gas drilling, influence the sub-surface microbiome(s)?

26 325 273. How will increasing urbanisation affect environmental and host-associated microbial
27 326 communities?

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

29 328 294. Can we manipulate microbial succession in species-poor soils to encourage repopulation by flora
30 329 and fauna?

31 330

32 331 **Environmental Processes**

33 332 Microbes play a fundamental role in environmental processes and ecosystem services, including
34 333 nutrient cycling and organic matter decomposition (Chin *et al.* 2016; Creamer *et al.*, 2015; Weider *et*
35 334 *al.*, 2013), bioremediation of contaminated habitats or waste systems (Haritash & Kaushik, 2009; Oller
36 335 *et al.*, 2011), and influencing greenhouse gas emissions (Singh *et al.*, 2010; Bragazza *et al.*, 2013; Hu
37 336 *et al.*, 2015). The ability to harness these processes has great potential for societal and environmental
38 337 applications, particularly in extremophiles, which frequently reveal metabolic capabilities and
39 338 evolutionary solutions not witnessed elsewhere in the microbial world (Coker *et al.* 2016). However, it
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
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.
14
15
16
17
18
19

20 347 [3025](#). How do we successfully establish microbial communities used in bioremediation?

21
22 348 [3126](#). How important is the rare microbiome in ecosystem function, and how does this change with
23 349 stochastic events?

24
25
26 350 [3227](#). To what extent is microbial community diversity and function resilient to short- and long-term
27 351 perturbations?

28
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?

31
32 354 [3449](#). How can we accurately measure microbial biomass in a reproducible manner?

33
34
35 355
36
37 356 [3529](#). Which mechanisms do extremophiles use for survival and how can they be exploited?
38 357

39 358 **Functional Diversity**

40
41
42
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
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7 366 biogeochemical processes. There is an urgent need to understand how the genome ~~or hologenome~~
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.
15
16
17
18 373

19
20 374 ~~369~~. What are the mechanisms driving microbial community structure and function, and are these
21 375 conserved across ecosystems?

22
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?

28
29 379 ~~392~~. What is the relative importance of individual 'species' for the functioning of microbial
30 380 communities?

31
32
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?

35
36
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?

42
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?"

46
47 389 4450. How useful are synthetic communities for testing~~inferring~~ theories about microbial community
48 390 dynamics and function?

49
50 391

51
52 392

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

393

394

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?

1
2
3
4
5
6 421 41. Can experimental evolution predict how antimicrobial resistance evolves *in vivo*?

Formatted: Font: Italic

7
8 422

9
10 423 ***Methods in Microbial Ecology***

11
12 424 Methods for assessing microbial diversity and community function have rapidly advanced in recent
13
14 425 years, with a major shift from culture dependent to molecular based techniques that produce vast
15
16 426 quantities of data (Rohwer, 2007; Bitten *et al.*, 2015). Advances in technologies for the analysis of
17
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
37
38 437 applications such as antibiotic discovery (Ling *et al.*, 2015; Oberhardt *et al.*, 2015). There is no one-
39
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.

43
44 443

45 444 42. What methods can we use to marry microbial diversity with function; how do we link
46
47 445 transcriptomics, proteomics and metabolomics?

48
49 446 43. How can we handle the unassigned sequences that dominate metagenome datasets?

50
51 447 44. How can we develop a standardized best-practice method for analysing sequence data to estimate
52
53 448 relative abundance?

54

55

56

57

58

59

60

- 1
2
3
4
5
6
7 449 ~~45. What is the most appropriate taxonomic level at which to compare microbial community~~
8 450 ~~compositions?~~
9
10 451 ~~46. How can systems approaches improve our understanding of host-microbe interactions?~~
11
12 452 ~~47. How do we move beyond correlations to determine cause and effect in microbial~~
13 453 ~~communities/ecosystems?~~
14
15
16 454 ~~48. How can we better track the source and dispersal of particular microorganisms in real time?~~
17
18 455 ~~49. How can we accurately measure microbial biomass in a reproducible manner?~~
19
20 456 ~~50. How useful are synthetic communities for inferring theories about microbial community dynamics?~~
21
22 457

23 24 458 **Society and Policy**

25
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.
32
33
34
35

- 36 465
37
38 466
 - 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?
 - How can we replace fear-based regulation with risk-based regulation, specifically with regard
42 470 to the use of microbes in bioremediation and bioaugmentation?

43 471
44 472
45 473
46
47
48
49
50
51
52
53 474
54
55
56
57
58
59
60

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

1
2
3
4
5
6
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
14
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
20
21 515 manipulated.

22
23
24
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).

35
36
37
38
39
40
41 526 Questions identified in this paper highlighted the need for knowledge that
42
43 527 informs antibiotic use and production, and to optimise the sustainability of food
44
45 528 production through improved soil fertility. There was a strong interest in maintaining
46
47 529 and enhancing the microbial populations of crop ecosystems, especially in light of
48
49 530 antibiotic resistance (Elouze *et al.*, 2014). As antibiotic resistance increases along with
50
51 531 our concern about potential impact on both human and animal health, there is an
52
53 532 increasing drive to find new forms of antibiotics.

Formatted: Normal, No widow/orphan control, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

1
2
3
4
5
6
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
9
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.

15
16 540 Global changes resulting from human activity impact almost every Earth habitat. It is
17
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.

33
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.

1
2
3
4
5
6
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~~
9
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
13 567 | ~~activities on microbial ecology, and to find new solutions to the environmental and health problems we~~
14
15 568 | ~~are currently facing and will continue to face in the future.~~

16
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,
19
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
22
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
27
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
30
31 579 | successful establishment of deliberately introduced organisms depends on an understanding of the
32
33 580 | principles underlying microbial community formation and structure (Rillig *et al.*, 2015). Despite these
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,
36 583 | and to what extent it is possible to lose species without affecting ecosystem functions. Already there is
37 584 | evidence that microbial communities may be less functionally redundant than macro-organism
38 585 | communities (Delgado-Baquerizo *et al.*, 2016). This issue ties into fundamental ecological concepts,
39 586 | such as niche theory (Carmona *et al.*, 2016); if multiple organisms are carrying out the same process,
40 587 | apparently interchangeably, how do they avoid competitively excluding one another? The concept of
41 588 | keystone species has been shown to be applicable to microbes (Neufeld *et al.*, 2008; Pester *et al.*,
42 589 | 2010; Ze *et al.*, 2012; Yu *et al.*, 2016), yet further work is needed to characterise the extent to which
43 590 | keystone functions occur in different environments and whether these can be consistently identified
44 591 | (Anderson, 2003; Pester *et al.*, 2010).

45
46
47
48
49
50
51
52
53
54 592 | ~~Recent major technological and analytical advancements have made previously inaccessible~~
55
56
57
58
59
60

1
2
3
4
5
6
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~~
9
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~~
12
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~~
15
16 599 ~~bacterial and fungal communities (Thomas et al., 2012; Smith & Peay, 2014), and bioinformatics of~~
17
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.~~

27
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
32 608 Policy' theme. Discussions included the benefits of forming collaborative and open research
33
34 609 communities, and the need to ensure the legacy of academic research through improving regulation
35
36 610 and policy and engagement with the public. Fear-based regulation of research, grounded in alarmist or
37
38 611 populist campaigns, as opposed to risk-based regulation built upon evidence, was identified as a
39
40 612 possible obstacle to progress, which could be addressed through greater interaction between
41
42 613 microbial ecologists and the public at both governmental and grass roots levels. Large scale
43
44 614 assessments of ecosystem services and degradation acknowledge the paucity of data on microbial
45
46 615 impacts, presumably because there are no convincing large-scale messages that can be derived at
47
48 616 this stage (Norris et al., 2011). Microbial diversity is therefore rarely considered when estimates of
49
50 617 biodiversity are required for policy or management decisions. That said, ~~the~~ increasing recognition of
51
52 618 the fundamental impact of the microbial world on the functioning of larger-scale processes has made
53
54 619 the deliberate manipulation of the microbial world a controversial subject, which was reflected in the
55
56 620 number of draft questions submitted related to bioremediation and bioaugmentation (see
57
58 621 Supplementary Information). Collaboration with social scientists was identified as crucial in gauging
59
60 622 the public understanding of microbial ecology, and citizen science approaches were considered as

Formatted: Normal, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

623 tools to tackle key microbial ecology research questions. ▲

Formatted: Font:

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
632 theory underpinning many research questions transcends scientific disciplines, and that there is still

Formatted: Font: English (U.K.)

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 gGiven 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

644 Funding

645 This work was supported by contributions from the British Ecological Society and the University of
646 Salford towards funding the workshop. SRJ is funded by NERC studentship NE/L501773/1, KMF by
647 the Finnish Cultural Foundation, NLMF is funded by Colciencias, MCM by Earth and Life Systems
648 Alliance, and WJS by Arcadia. The positions and opinions presented in this article are those of the
649 authors alone and are not intended to represent the views or scientific works of the European Food
650 Safety Authority.

651

1
2
3
4
5
6 652 **Acknowledgements**
7

8
9 653 Our thanks go to Francis Brearley and Michael Cunliffe for their submissions to the pre-workshop
10 654 questions, and to Jessica Hall, Daniel Henk and Matt Lloyd Jones for their contributions to the
11 655 workshop.
12
13 656

14
15
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
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
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

Formatted: Font: Spanish (International Sort)

- 1
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
12 710 sustainability of agriculture? A prioritisation of knowledge needs for the UK food system. *Sustainability*,
13 711 2013;**5**, 3095–3115
- 16 712 Dumont, MG, Murrell, JC. Community-level analysis: key genes of aerobic methane oxidation.
17 713 *Methods Enzymol*, 2015;**397**, 413-427.
- 20 714 El Khawand, M, Crombie, AT, Johnston, A, *et al.* Isolation of isoprene degrading bacteria from soils,
21 715 development of isoA gene probes and identification of the active isoprene degrading soil community
22 716 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
26 718 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
30 720 enough. *Systematic Biology*, 2016;10.1093
- 32 721 Fox, JL. Agricultural probiotics enter spotlight. *Nature Biotechnology*, 2015;**33**, 122
- 34 722 Franklin AM, Aga, DS, Cytryn, E, *et al.* Antibiotics in agroecosystems: Introduction to the special
35 723 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
39 725 microbial community profiling. *Nat. Rev. Microbiol*, 2015;**13**, 360-372
- 41 726 Fredriksson, N, Hermansson, M, Wilén, B The choice of PCR primers has great impact on
42 727 assessments of bacterial community diversity and dynamics in a wastewater treatment plant. *PLoS*
43 728 *One*, 2013;**8**, e76431
- 46 729 Haritash, AK., Kaushik, CP Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A
47 730 review. *J Hazard Mater*, 2009;**169**, 1-15
- 50 731 Hartmann, M, Frey, B, Mayer, J, *et al.* Distinct soil microbial diversity under long-term organic and
51 732 conventional farming. *ISME J*, 2015;**9**, 1177-1194

Formatted: Font: French (France)

Formatted: Font: German (Germany)

Formatted: Font: Spanish (International Sort)

Formatted: Font: Spanish (International Sort)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
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
55
56
57
58
59
60

Formatted: Font: French (France)

Formatted: Font: English (U.K.)

Formatted: Font: French (France)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

Formatted: Font: French (France)

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

Formatted: Font: French (France)

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

Formatted: Font: French (France)

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

Formatted: Font: French (France)

- 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
52
53
54
55
56
57
58
59
60

Formatted: Font: Spanish (International Sort)

Formatted: Font: French (France)

Formatted: Font: Spanish (International Sort)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Formatted: Font: English (U.K.)

Formatted: Font: English (U.K.)

Formatted: English (U.K.)

- 1
2
3
4
5
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
48
49
50
51
52
53
54
55
56
57
58
59
60

Formatted: Font: French (France)

Formatted: Font: French (France)

- 1
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, the
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?
- 45
- 46
- 47
- 48
- 49
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

- 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 How do protists influence fungi in soil?
- 25 How do skin microbiota and UV interact in amphibians, especially at high altitude?
- 26 How do soil bacteria, viruses and archaea interact?
- 27 How do virus influence microbial ecology?
- 28
- 29 How do we establish effective sample sizes for studies of poorly characterised microorganism?
- 30 How do we sample environmental variables at an appropriate scale that are proximate to microbial comr
- 31 How does individual microbiota affect others at a community level
- 32 How does the environment select?
- 33 How does the microbiome influence health?
- 34 How important is the "rare" microbiome in ecosystem function?
- 35 How important is the microbiota in mediating adaptation that results in species invasions?
- 36 How is best to study the changing resource environment of microbial communities?
- 37 How is climate change going to affect the microbial communities in the drinking water distribution system
- 38 How much functional redundancy is there in microbes relative to higher taxa?
- 39 How much functional redundancy is there in microbial communities, and how does that interact with divi
- 40 How much functional redundancy is there in the host microbiome?
- 41 How much intra- vs inter-specific trait variation is there?
- 42 How much microbial diversity in the soil has been lost through monoculture and what is the importance (
- 43 How problematic is PCR amplification bias?
- 44 How similar are is the rhizosphere biodiversity of microbes from the same species of tree located in differ
- 45 How stable are microbial communities, and how can we assess their stability, particularly in changing env
- 46 How to foster interdisciplinary approaches in and around microbial ecology?
- 47 How to solve the leaky pipeline of female microbial ecologists in academia?
- 48 How to solve the peer reviewer crisis in microbial ecology?
- 49 How well do current species distribution models developed with microbial systems predict the distributic
- 50
- 51
- 52
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

1
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 enviro
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?
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
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
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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?
n?
?

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?

1
2 diation?
3 capacities, genomes)?
4 (ane, etc)? What are the implications of microbial species loss in these cycles?
5
6 evolution?
7
8
9

10 microbial communities or do this methods get bias from collecting too much material from dead
11
12
13
14
15
16
17
18
19
20
21

22 root, size of the whole tree, maturity of the tree?
23
24

25 nunity in streams?
26
27

28 r microbiota?
29 re presumably barophilic?
30
31

32 n experimental artifacts?
33
34

35 eld?
36
37
38
39
40
41
42
43
44

45 making bodies
46 sity in soil microbial ecosystems?
47
48
49
50
51
52
53
54
55
56
57

58 se ~big data~ projects?
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2 f microbiota of umbrella species?

3
4 he faecal matter of livestock and deposit on the land. What will be the long term effect on micro
5
6
7

8
9
10
11
12 effort into virus and microeukaryote ecology?

13
14
15
16 organisms?

17
18
19 is ecosystems (e.g. marine, freshwater, terrestrial, gut microbiota)?

20
21
22
23 e more easily published than negative ones) in microbial ecology?

24
25
26
27
28
29
30 iome environment?

31
32
33 effects?

34
35
36
37
38 iudies of microbial ecology?

39
40
41 less on documenting diversity?

42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
ormation about the abiotic factors of a soil?
ences mean anything?

ut the years we have established the importance of temperature, pH, moisture, basic factorsâ€¦
of any resulting microbiome shift for agriculture and biodiversity?

ystem?

urring within an individual's microbiome?

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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?

For Peer Review

1
2
3
4
5
6
7
8
9
10 ;?
11
12
13
14
15
16
17
18
19 decades?
20
21
22
23
24
25 rine organisms?
26 tosanitary regulators?
27 d the rise of metabolomic and transcriptomic studies
28 d communication problems within the field?
29 pean ash dieback?
30 r is the recent surge in the UK a short-lived burst?
31
32
33
34
35
36
37
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
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

cells?

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

?

For Peer Review

1
2
3
4
5 microbial population as a driver for antibiotic resistance?
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53 which are the new most important factors that are of interest to microbial ecologists?
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

onments?

For Peer Review