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50 important research questions in microbial ecology

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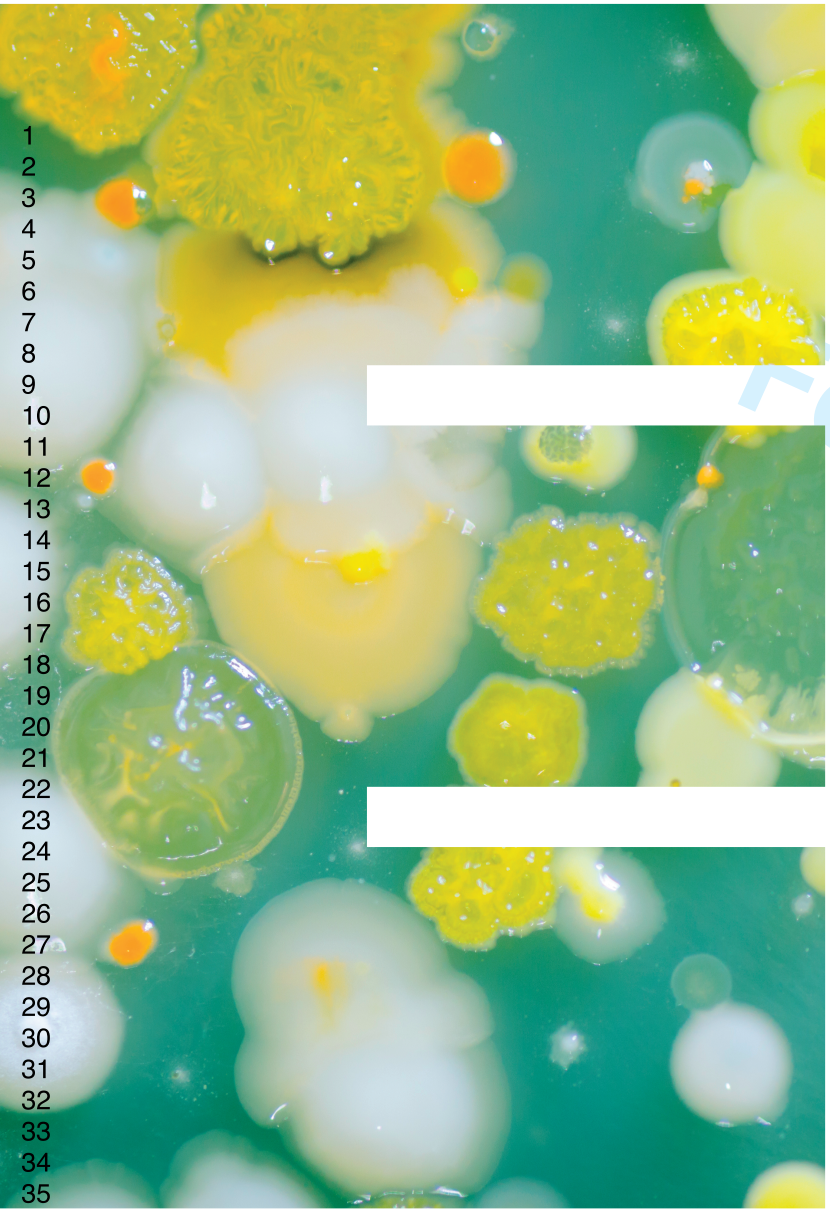

MICROBIAL
ECOLOGY
GROUP

50 important research questions in microbial ecology

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Abstract

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

Introduction

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

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

Methods

Participants

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

British Ecological Society's membership mailing list and through social media (Twitter and Facebook). In total, 34 participants from 20 institutions attended and contributed to the development of the 50 questions listed below, with the majority listed as authors on this paper.

Questions

Prior to the workshop, attendees were asked to submit questions via an online form that they thought most closely met the following brief:

> "We are aiming to identify 50 questions that, if answered, will make a considerable difference to the use of microbial ecology by practitioners and policy makers, or to the fundamentals of the field of microbial ecology. These should be questions that are unanswered, could be answered, and could be tackled by a research programme. This is expected to set the agenda for future research in the field of microbial ecology."

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

1) *Host-Microbiome Interactions;*
2) *Health and Infectious Diseases;*
3) *Human Health and Food Security;*
4) *Microbial Ecology in a Changing World;*
5) *Environmental Processes;*
6) *Functional Diversity;*
7) *Evolutionary Processes.*

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

Question selection process

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

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

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

Limitations

All but four participants were from British universities, although there were representatives from a range of nationalities and research areas. The manner in which this paper was developed (*i.e.* through a physical workshop and via the British Ecological Society) means that, without a substantial travel budget, a bias towards UK institutions was inevitable. However, many participants have worked on, or currently collaborate in, research projects on non-UK ecosystems and species, and therefore the questions proposed are drawn from considerable knowledge and experience of the field internationally. Additionally, although most individuals were from academic institutions, many individuals had previous or on-going collaborations with industrial partners and governmental/non-governmental organisations.

Results

The following 50 questions are presented by theme, and are not ordered according to relevance or importance. Due to the nature of the process, some questions may appear similar across themes, but within the context of each theme can take on a different meaning. Some questions may relate to research areas that are already somewhat active, and these serve to highlight the importance of and encourage further work in these areas. Some of these questions apply across multiple biomes and ecosystems, and can be considered in the context of multiple host organisms and across varying temporal and spatial scales.

Host-Microbiome Interactions

Host-microbiome interactions determine many host life history traits such as behaviour, reproduction, physiological processes, and disease susceptibility (Archie and Theis, 2011; Willing *et al.,* 2011; Koch & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.,* 2016). Increasingly, we are discovering that host-microbiome interactions produce complex and dynamic communities that fluctuate in compositional abundance correlated with factors as diverse as host genotype, developmental stage, diet, and temporal changes, among others (e.g. Spor *et al*., 2011). Even in otherwise well studied organisms, very little is known about the consequences of microbiome variation for host processes, particularly across different spatial and temporal scales. Considerations of host microbiomes are also likely important for global issues, such as the efficacy of conservation efforts including species reintroduction programmes (reviewed in Redford *et al*., 2012; McFall-Ngai, 2015). Additionally, interactions between native and non-native species are correlated with transmission of microbiota, often determined by relatedness or diet type (Ley *et al.,* 2008), and the microbiome plays a key role in the control and competence of insect crop pests and vectors of disease (reviewed in Weiss & Aksoy, 2011). The following questions aim to address the shortfall in our understanding of the interactions between microbiomes and their human and non-human hosts.

1.What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe interactions?

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

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

4. Can compositional or evolutionary changes in microbiomes help hosts adapt to environmental change within the lifetime of the host?

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

6. How can the associated microbiota be effectively included in risk assessments of Invasive Non-Native Species?

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

8. How can a ‘systems biology’ approach improve our understanding of host-microbe interactions?

Health and Infectious Diseases

The last 50 years have seen the emergence of several hypervirulent wildlife pathogens in animals (*e.g*. Tasmanian devil face tumour disease, avian malaria, amphibian chytridiomycosis; reviewed in Tompkins *et al*., 2015) and plants (e.g. sudden oak and larch death, ash dieback; Pautasso *et al*., 2015). Although the role of microorganisms as pathogens is well known, the importance of host-associated microbiomes in regulating disease susceptibility is becoming more apparent (Koch & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.,* 2016). A major outstanding research goal is to understand how within-host interactions among microbes and invading pathogens may shape patterns of infection intensity and disease progression (see also *Evolutionary Processes*). Several studies have sought to determine how manipulation of host microbiomes may ameliorate the spread and impact of such diseases (*e.g.* Rebollar *et al.,* 2016).

While for many disease states the paradigm holds true that one microorganism causes one disease, polymicrobial infections are becoming more apparent through metagenomic and metatranscriptomic sequencing of disease-associated microbial communities (Gilbert *et al.,* 2016). Consequently, the ’pathobiome‘ concept, where a disease state is influenced by complex interactions between commensal and pathogenic microorganisms, presents new challenges for applying Koch’s postulates to diseases arising from polymicrobial interactions (Vayssier-Taussat *et al.,* 2014), such as black band disease (BBD) in corals (Sato *et al.*, 2016) and olive knot disease (Buonaurio *et al.,* 2015).

In this theme we have identified research questions relating to the microbial ecology of infectious diseases and host health. Although much can be learnt from the comparatively high number of studies in the human and biomedical literature (*e.g.* using network approaches in epidemiology), the questions selected in this theme predominantly relate to non-human animals and plants, as humans are covered later (‘*Human Health and Food Security*’).

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

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

11. Which factors trigger ‘covert’ infections to become ‘overt', impacting host health?

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

13. What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms of their survival and spread?

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

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

Human Health and Food Security

With the human population due to exceed eight billion by 2024, food security and human health are high on political and scientific agendas. The human microbiome has been the focus of intense research efforts in recent years, (e.g. Walter & Ley, 2011; Spor *et al.,* 2011; Mueller *et al.,* 2012), because gut symbionts shape the immune response (Round *et al*., 2009), and diversity fluctuates through chronic conditions and infectious diseases including diabetes, obesity (Serino et al. 2016; Baothman *et al.,* 2016; Ridaura *et al.,* 2013), asthma (Smits et al. 2016), and HIV (Lozupone *et al.,* 2013). Improving our understanding of the core human microbiome and individual variation will underpin pharmomicrobiomics, enabling development of novel therapeutic treatments and, ultimately, personalised medicine (e.g. Ubeda *et al.,* 2013).

Antibiotic resistance resulting from selective pressures generated by the use and misuse of antibiotics is a global threat to public health (Levy, 1997; Tam *et al*., 2012). The volume of antibiotics used in agriculture now exceeds the amount used in human medicine in many countries (WHO, 2011). Antibiotics are still widely used in livestock for prophylaxis and growth promotion, often at sub-therapeutic concentrations, exacerbating resistance (Krishnasamy *et al*., 2015). The impact of the leaching of antibiotics into the natural environment and subsequent impacts on natural microbial communities remains poorly characterised (Franklin *et al.,* 2016). Current practices of growing high-intensity monoculture crops have a negative impact on the microbial biodiversity of soils through a combination of tillage, subsequent erosion and chemical applications (Helgason *et al*., 1998; Jacobsen and Hjelmsø, 2014; Zuber and Villamil, 2016), which imposes selection pressures on pathogenic microbes, fungal symbiotic partners and plant growth promoting bacteria (Chapparo *et al.,* 2012; Hartmann *et al.,* 2015). Thus, there is a need to maintain and enhance microbial populations of crop ecosystems, especially in light of antibiotic resistance (Ellouze *et al*., 2014). As antibiotic resistance increases, along with our concern about potential impact on both human and animal health, there is an increasing drive to find new forms of antibiotics.

Though the remit for this section is relatively broad, the questions focus on two central themes: i) studying the human microbiome to improve the treatment of disease, including the development of personalized medicine and novel antibiotics; and ii) understanding how *current* antibiotic regimes and farming practices may negatively impact the diversity of the environmental microbiome and food production capacity.

16. How can human microbiome studies improve personalised medicine?

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

18. What are the main determinants of waterborne infection outbreaks, and what is the best strategy to control these in water distribution systems?

19. What are the consequences of antibiotic and pharmaceutical use in human medicine on microbial communities in freshwater and soil environments?

20. To what extent are microbial species distributions influenced by climate, and what are the consequences for food security and human health?

21. How much microbial diversity in the soil has been lost through monoculture and what is the importance of this?

22. Intensive farming may involve high levels of agrochemicals and broad-spectrum antibiotic usage - what will be the long-term effects on microbial communities?

23. How best can we harness microbial communities to enhance food production?

Microbial Ecology in a Changing World

Global changes resulting from human activity impact almost every habitat on earth. It is imperative that we focus efforts on understanding the impacts of human activities such as climate change, urbanisation, agriculture, and industrial processes on microbial communities, ecosystem functioning equilibrium, and host health. Microbial populations have a tremendous capacity to adapt to changes in their abiotic environment, yet the functional implications of these transitions in microbial ecology are still poorly understood and characterised (Bissett *et al.*, 2013), and the role of microbes in mediating the response of larger organisms to change is equally understudied. Global environmental changes (GECs) are complex and multifaceted. Human activities such as urbanisation, land-use change and introduction of invasive species have played a role in shifting global ecosystems via desertification, climate change and habitat degradation. Although such changes have been quantified in aquatic and terrestrial habitats (*e.g.* Haberl *et al.*, 2007; Halpern *et al.*, 2008), their effects on microbial communities and impacts on ecosystem function are often hindered by a lack of characterisation of communities, or limited understanding of microbial functional traits. Shifts in basic nutrients and gases such as CO_2, along with temperature fluctuations and water availability, greatly influence the distribution and behaviour of species (Tylianakis *et al.,* 2008). GECs can alter host fitness or ecosystem functioning (Shay *et al.,* 2015; Webster *et al.* 2016) and are likely to occur in combination. While there is a great deal of research into the effects of each of these on microbial communities (Schimel *et al.,* 2007; Shurin *et al.,* 2012; Lloret *et al.,* 2014), literature considering the effect of multiple GECs is sparser, and these have complicated and often unpredictable consequences when combined (although see Hutchins *et al.,* 2009; Ryalls *et al.,* 2013). In this section, we consider how human activities directly and indirectly influence the microbial world. Where applicable, these questions can be considered across multiple biomes and ecosystems, with reference to resulting trophic cascades, in addition to the impacts on multiple biogeochemical processes. We also consider how microbes can be used as a tool for mitigation or bioremediation of human-induced environmental changes, and the ways in which microbes can be included in current evaluations of global change.

24. How can we integrate microbial communities into models of global change?
25. Will ocean acidification, temperature increases and rising sea levels lead to changes in microbial diversity or function, and what will the cascading effects of this be?
26. How do human activities, such as oil and gas drilling, influence the sub-surface microbiome(s)?
27. How will increasing urbanisation affect environmental and host-associated microbial communities?
28. How resilient are different microbial functional groups to ecosystem disturbance?
29. Can we manipulate microbial succession in species-poor soils to encourage repopulation by flora and fauna?

Environmental Processes

Microbes play a fundamental role in environmental processes and ecosystem services, including nutrient cycling and organic matter decomposition (Chin et al. 2016; Creamer *et al*., 2015; Weider *et al.,* 2013), bioremediation of contaminated habitats or waste systems (Haritash & Kaushik, 2009; Oller *et al.,* 2011), and influencing greenhouse gas emissions (Singh *et al.,* 2010; Bragazza *et al.,* 2013; Hu *et al*, 2015). The ability to harness these processes has great potential for societal and environmental applications, particularly in extremophiles, which frequently reveal metabolic capabilities and evolutionary solutions not witnessed elsewhere in the microbial world (Coker et al. 2016). However, it is rarely possible to directly link the presence of a specific microbial taxon to a particular ecological process. Other methodological challenges include establishing the relative importance of biotic and abiotic factors in microbial ecosystem function, and determining the appropriate spatial and temporal scale necessary to discriminate links between microbiota and their ecological functions (Bissett *et al*., 2013). Concurrently, a deeper understanding is required of human-induced impacts on the global microbiome through urbanisation, habitat degradation, climate change, and the introduction of invasive species, amongst others.

30. How do we successfully establish microbial communities used in bioremediation?

31. How important is the rare microbiome in ecosystem function, and how does this change with stochastic events?

32. To what extent is microbial community diversity and function resilient to short- and long-term perturbations?

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

34. How can we accurately measure microbial biomass in a reproducible manner?

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

Functional Diversity

Ecologists are increasingly turning their attention to classifying species based on their activity (function) within an ecosystem, rather than their genotype (Crowther *et al.,* 2014). This is particularly relevant for microbial ecology, in which species are hard to define, horizontal gene transfer is rife, and taxonomy is often blurred. Understanding how membership within complex and dynamic microbial communities relates to the function of that community is one of the key challenges facing microbial ecology (Widder *et al*., 2016). This is true across a vast range of spatial scales, from microbial dyads to the gut of a *Drosophila* fly, to ancient trees and their associated ecosystems, right through to global biogeochemical processes. There is an urgent need to understand how the genome of a microbial community (and in some cases, its host) relates to metabolic capacities. Conversely, there is also a need to understand how ecosystems depend on a particular organism or group of organisms for any given process and function. This section describes the need to move from simply describing microbial diversity to understanding what these organisms are doing, how they are doing it, and what biotic and abiotic drivers are controlling their activity. Each question may derive a suite of different answers, depending on the group of organisms, the habitat and the process.

36. What are the mechanisms driving microbial community structure and function, and are these conserved across ecosystems?

37. What is the relative importance of stochastic vs. determinative processes in microbial community assembly?

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

39. What is the relative importance of individual ‘species’ for the functioning of microbial communities?

40. How much functional redundancy is there in microbial communities, and how does functional redundancy affect measures of diversity and niche overlap?

41. How often are functional traits of microbes successfully conferred through horizontal gene transfer?

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

43. How do we move beyond correlation to develop predictive models that advance our understanding of microbial community function and dynamics?”

44. How useful are synthetic communities for testing theories about microbial community dynamics and function?

Evolutionary Processes

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

45. How can a bacterial 'species' be defined?
46. To what extent is faunal and floral biodiversity influenced by microbial communities?
47. To what extent do microbial communities have an equivalent to keystone ‘species’?
48. Does the structure of microbial communities conform to the same ecological rules/principles as in other types of communities?
49. How do fundamental shifts in environmental conditions impact the trajectory of microbial evolution?
50. What are the relative selective forces favouring microbial genome expansion or reduction?

Society and Policy

We need to find ways to apply fundamental biological research to the benefit of society and policy. For example, collaboration with social scientists is crucial when investigating public understanding of microbial ecology, as well as using citizen science approaches to tackle microbial ecology research questions. Many questions relating to this area were discussed at the workshop, and here we present four additional questions that were developed at the meeting that relate to societal and policy-based aspects of microbial ecology.

- How can we best address supply and demand of information about microbial ecology between researchers, clinicians, policy makers and practitioners?
- How can we best use social and traditional mass media for early identification of emerging threats to animal and plant health?
- How can we develop an open access data repository or integrate existing databases to create 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 to the use of microbes in bioremediation and bioaugmentation?

Discussion

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

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

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

Questions considered in both the '*Functional Diversity'* theme and the '*Environmental Processes'* theme raised a common need to understand changes in microbial community structure and function across spatial and temporal scales (Carmona *et al.*, 2016). Establishing appropriate spatial scales for studying microbial processes is an outstanding challenge: micro-organisms can orchestrate ecosystem functioning across whole biomes (Sheffer *et al.*, 2015), yet fungi exhibit low mobility on tree barks (Koufopanou *et al*. 2006, Robinson *et al.,* 2016), and an air void in soil can be an insuperable barrier for a bacterium. Similarly, drawing meaningful conclusions about microbial processes requires understanding of their temporal variability; for example, diurnal influences (Shurpali *et al.,* 2016), or lags behind changes in ecosystem drivers (Allison and Martiny, 2008).

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

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

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

The 50 questions identified here cover a broad range of topics, but some over-arching themes recur across multiple questions, including a recognition that microbes play an important role in a variety of different processes and systems, which may be harnessed to solve real-world problems. There were some similarities between the questions identified here and those identified by previous workshops of a similar nature. For example, questions relating to soil health and biodiversity (Dicks *et al.* 2013), a requirement for developing a theoretical understanding of micro- and macro- ecological concepts (Prosser et al. 2007, Sutherland et al. 2013a) and disease dynamics (Prosser et al. 2007, Sutherland et al. 2013a) have a degree of commonality with this list. This indicates that the ecological theory underpinning many research questions transcends scientific disciplines, and that there is still much work to be done at both theoretical and applied levels. Within these 50 questions, we have tried to provide a focus for researchers addressing scientific questions from a microbial perspective, regardless of their background. It is expected that these questions will facilitate interesting discussion and new, exciting, interdisciplinary research. The list is by no means exhaustive, and we recognise that the questions presented here are relatively community-centric, primarily due to the recent expansion in methodological approaches that have improved our understanding of microbial community diversity and function. That said, other areas of microbial ecology should not be ignored or forgotten. Given the rapidly evolving field of microbial ecology, it is expected that future workshops with a wide draw will be held to ensure that the identification of research priorities and areas of interest is a continuing process.

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50 important research questions in microbial ecology

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environmental processes, evolutionary processes, functional diversity, host-microbiome interactions, ~~methods,~~ priority setting, research agenda

Abstract

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

Introduction

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

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

Methods

Participants

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

British Ecological Society's membership mailing list and through social media (Twitter and Facebook). In total, 34 participants from 20 institutions attended and contributed to the development of the 50 questions listed below, with the majority listed as authors on this paper.

Questions

Prior to the workshop, attendees were asked to submit questions via an online form that they thought most closely met the following brief:

> "We are aiming to identify 50 questions that, if answered, will make a considerable difference to the use of microbial ecology by practitioners and policy makers, or to the fundamentals of the field of microbial ecology. These should be questions that are unanswered, could be answered, and could be tackled by a research programme. This is expected to set the agenda for future research in the field of microbial ecology."

A total of 244 questions were submitted by attendees (see Supplementary Information), and assigned (by R.E. Antwis and S.M. Griffiths) to the following ~~eight~~ themes;

1) *Host-Microbiome Interactions;*
2) *Health and Infectious Diseases;*
3) *~~Food Security and~~ Human Health and Food Security;*
4) *Microbial Ecology in a Changing World;*
5) *Environmental Processes;*
6) *Functional Diversity;*
7) *Evolutionary Processes.~~;~~*
8) ~~*Methods in Microbial Ecology.*~~

An additional ~~ninth~~ eighth theme named '*Society and Policy'* was created to capture a number of questions that were generally applicable across the biological sciences, as well as a number of questions specific to the field of microbial ecology which could not necessarily be addressed through laboratory based microbial ecology research, *per se*.

Question selection process

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

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

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

Limitations

All but four participants were from British universities, although there were representatives from a range of nationalities and research areas. The manner in which this paper was developed (*i.e.* through a physical workshop and via the British Ecological Society) means that, without a substantial travel budget, a bias towards UK institutions was inevitable. However, many participants have worked on, or currently collaborate in, research projects on non-UK ecosystems and species, and therefore the questions proposed are drawn from considerable knowledge and experience of the field internationally. Additionally, although most individuals were from academic institutions, many individuals had previous or on-going collaborations with industrial partners and governmental/non-governmental organisations.

Results

The following 50 questions are presented by theme, and are not ordered according to relevance or importance. Due to the nature of the process, some questions may appear similar across themes, but within the context of each theme can take on a different meaning. Some questions may relate to research areas that are already somewhat active, and these serve to highlight the importance of and encourage further work in these areas. Some of these questions apply across multiple biomes and ecosystems, and can be considered in the context of multiple host organisms and across varying temporal and spatial scales.

Host-Microbiome Interactions

Host-microbiome interactions determine many host life history traits such as behaviour, reproduction, physiological processes, and disease susceptibility (Archie and Theis, 2011; Willing *et al.,* 2011; Koch & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.,* 2016). ~~The 'hologenome theory' suggests that the microbiome be considered an integral part of the host system, with the evolution of an individual's own genetic material and that of the associated microbiota intrinsically linked (Zilber-Rosenberg & Rosenberg, 2008; Daskin & Alford, 2012).~~ Increasingly, we are discovering that host-microbiome interactions produce complex and dynamic communities that fluctuate in compositional abundance ~~influenced by~~correlated with factors as diverse as host genotype, developmental stage, diet, and temporal changes, among others (e.g. Spor *et al*., 2011). Even in otherwise well studied organisms, very little is known about the consequences of microbiome variation for host processes, particularly across different spatial and temporal scales. Considerations of host microbiomes are also likely important for global issues, such as the efficacy of conservation efforts including species reintroduction programmes (reviewed in Redford *et al*., 2012; McFall-Ngai, 2015). Additionally, interactions between native and non-native species are ~~affected by~~correlated with transmission of microbiota, often determined by relatedness or diet type (Ley *et al.,* 2008), and the microbiome plays a key role in the control and competence of insect crop pests and vectors of disease (reviewed in Weiss & Aksoy, 2011). The following questions aim to address the shortfall in our understanding of the interactions between microbiomes and their human and non-human hosts.

1.~~1.~~ What are the primary mechanisms within a host that mediate microbe-microbe and host-microbe interactions?

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

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3~~2~~. How do microbial communities function to affect the phenotype of the host?

4~~3~~. Can compositional or evolutionary changes in microbiomes help hosts adapt to environmental change within the lifetime of the host?

5~~4~~. What is the role of the microbiota in host speciation processes?

6~~5~~. How can the associated microbiota be effectively included in risk assessments of Invasive Non-Native Species?

7~~6~~. How does the microbiome of captive ~~and head-started~~ animals affect the success of reintroduction programmes?

8. How can a ‘systems biology’ approach improve our understanding of host-microbe interactions?

Health and Infectious Diseases

The last 50 years have seen the emergence of several hypervirulent wildlife pathogens in animals (*e.g.* Tasmanian devil face tumour disease, avian malaria, amphibian chytridiomycosis; reviewed in Tompkins *et al*., 2015) and plants (e.g. sudden oak and larch death, ash dieback; Pautasso *et al*., 2015). Although the role of microorganisms as pathogens is well known, the importance of host-associated microbiomes in regulating disease susceptibility is becoming more apparent (Koch & Schmidt-Hempel, 2011; Daskin & Alford, 2012; King *et al.,* 2016). A major outstanding research goal is to understand how within-host interactions among microbes and invading pathogens may shape patterns of infection intensity and disease progression (see also *Evolutionary Processes*). Several studies have sought to determine how manipulation of host microbiomes may ameliorate the spread and impact of such diseases (*e.g.* Rebollar *et al.,* 2016).

While for many disease states the paradigm holds true that one microorganism causes one disease, polymicrobial infections are becoming more apparent through metagenomic and metatranscriptomic sequencing of disease-associated microbial communities (Gilbert *et al.,* 2016). Consequently, the “~~‘~~pathobiome”~~’~~ concept, where a disease state is influenced by complex interactions between commensal and pathogenic microorganisms, presents new challenges for applying Koch’s postulates to diseases arising from polymicrobial interactions (Vayssier-Taussat *et al.,* 2014), such as black band disease (BBD) in corals (Sato *et al.*, 2016) and olive knot disease (Buonaurio *et al.,* 2015).

In this ~~section~~ theme we have identified research questions relating to the microbial ecology of infectious diseases and host health. Although much can be learnt from the comparatively high number of studies in the human and biomedical literature (*e.g.* using network approaches in epidemiology), the questions selected in this theme predominantly relate to non-human animals and plants, as humans are covered later (‘~~*Food Security and*~~ *Human Health and*~~’~~ *Food Security’*).

9~~48~~. How can we better track the source and dispersal of particular microorganisms in real time?

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10~~7~~. Many microorganisms are unculturable, and many microbiome studies reveal that diseases are polymicrobial; how can we re-evaluate Koch's postulates in this context?

11~~8~~. Which factors trigger ‘covert’ infections to become ‘overt', impacting host health?

12~~9~~. At the population level, how is the burden and shedding intensity of intracellular microbes affected by co-infection by extracellular parasites?

13~~10~~. What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms of their survival and spread?

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

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

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

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

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

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

Antibiotic resistance resulting from selective pressures generated by the use and misuse of antibiotics is a global threat to public health (Levy, 1997; Tam *et al.*, 2012). The volume of antibiotics used in agriculture now exceeds the amount used in human medicine in many countries (WHO, 2011). Antibiotics are still widely used in livestock for prophylaxis and growth promotion, often at sub-therapeutic concentrations, exacerbating resistance (Krishnasamy *et al*., 2015). The impact of the leaching of antibiotics into the natural environment and subsequent impacts on natural microbial communities remains poorly characterised (Franklin *et al.,* 2016). Current practices of growing high-intensity monoculture crops have a negative impact on the microbial biodiversity of soils through a combination of tillage, subsequent erosion and chemical applications (Helgason *et al*., 1998; Jacobsen and Hjelmsø, 2014; Zuber and Villamil, 2016), which imposes selection pressures on pathogenic microbes, fungal symbiotic partners and plant growth promoting bacteria (Chapparo *et al.,* 2012; Hartmann *et al.,* 2015). Thus, there i~~was~~ a ~~strong interest in~~need to maintain~~ing~~ and enhance~~ing~~ ~~the~~ microbial populations of crop ecosystems, especially in light of antibiotic resistance (Ellouze *et al.*, 2014). As antibiotic resistance increases, along with our concern about potential impact on both human and animal health, there is an increasing drive to find new forms of antibiotics.

Though the remit for this section is relatively broad, the questions focus on two central themes: i) studying the human microbiome to improve the treatment of disease, including the development of personalized medicine and novel antibiotics; and ii) understanding how *current* antibiotic regimes and farming practices may negatively impact the diversity of the environmental microbiome and food production capacity.~~This section examines ways in which we can harness microbial functions to improve overall human health through managing gut microbiota, and improve the soil and plant microbiome, thus increasing yields and associated biodiversity.~~

16~~3~~. How can human microbiome studies improve personalised medicine?

17~~4~~. What ecological principles can be applied in the search for new antibiotics and alternatives?

18~~5~~. What are the main determinants of waterborne infection outbreaks, and what is the best strategy to control these in water distribution systems?

19~~6~~. What are the consequences of antibiotic and pharmaceutical use in human medicine on microbial communities in freshwater and soil environments?

20. To what extent are microbial species distributions influenced by climate, and what are the consequences for food security and human health?

21~~17~~. How much microbial diversity in the soil has been lost through monoculture and what is the importance of this?

22~~18~~. Intensive farming may involve high levels of agrochemicals and broad-spectrum antibiotic usage - what will be the long-term effects on microbial communities?

23~~19~~. How best can we harness microbial communities to enhance food production?

Microbial Ecology in a Changing World

Global changes resulting from human activity impact almost every ~~Earth~~ habitat on earth. It is imperative that we focus efforts on understanding the impacts of human activities such as climate change, urbanisation, agriculture, and industrial processes on microbial communities, ecosystem functioning equilibrium, and host health. Microbial populations have a tremendous capacity to adapt to changes in their abiotic environment, yet the functional implications of these transitions in microbial ecology are still poorly understood and characterised (Bissett *et al.*, 2013), and the role of microbes in mediating the response of larger organisms to change is equally understudied. ~~Plant-associated microbial communities can alter the performance of subsequent generations of plants, and can themselves be subject to the concomitant effects of abiotic change (van der Putten *et al.*, 2013). Such plant-soil feedbacks can reduce yield and alter the community composition and invasibility of whole areas of grassland (van der Putten *et al.*, 2013).~~ Global environmental changes (GECs) are complex and multifaceted. Human activities such as urbanisation, land-use change and introduction of invasive species have played a role in shifting global ecosystems via desertification, climate change and habitat degradation. Although such changes have been quantified in aquatic and terrestrial habitats (*e.g.* Haberl *et al.*, 2007; Halpern *et al.*, 2008), their effects on microbial communities and impacts on ecosystem function are often hindered by a lack of characterisation of communities, or limited understanding of microbial functional traits. Shifts in basic nutrients and gases such as CO_2, along with temperature fluctuations and water availability, greatly influence the distribution and behaviour of species (Tylianakis *et al.,* 2008). GECs can alter host fitness or ecosystem functioning (Shay *et al.,* 2015; Webster *et al.* 2016) and are likely to occur in combination. While there is a great deal of research into the effects of each of these on microbial communities (Schimel *et al.,* 2007; Shurin *et al.,* 2012; Lloret *et al.,* 2014), literature considering the effect of multiple GECs is ~~more sparse~~sparser, and these have complicated and often unpredictable consequences when combined (although see Hutchins *et al.,* 2009; Ryalls *et al.,* 2013). In this section, we consider how human activities directly and indirectly influence the microbial world. Where applicable, these questions can be considered across multiple biomes and ecosystems, with reference to resulting trophic cascades, in addition to the impacts on multiple biogeochemical processes. We also consider how microbes can be used as a tool for mitigation or bioremediation of human-induced environmental changes, and the ways in which microbes can be included in current evaluations of global change.

24~~0~~. How can we integrate microbial communities into models of global change?

25~~1~~. Will ocean acidification, temperature increases and rising sea levels lead to changes in microbial diversity or function, and what will the cascading effects of this be?

26~~2~~. How do human activities, such as oil and gas drilling, influence the sub-surface microbiome(s)?

27~~3~~. How will increasing urbanisation affect environmental and host-associated microbial communities?

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

29~~4~~. Can we manipulate microbial succession in species-poor soils to encourage repopulation by flora and fauna?

Environmental Processes

Microbes play a fundamental role in environmental processes and ecosystem services, including nutrient cycling and organic matter decomposition (Chin et al. 2016; Creamer *et al*., 2015; Weider *et al.,* 2013), bioremediation of contaminated habitats or waste systems (Haritash & Kaushik, 2009; Oller *et al.,* 2011), and influencing greenhouse gas emissions (Singh *et al.,* 2010; Bragazza *et al.,* 2013; Hu *et al*, 2015). The ability to harness these processes has great potential for societal and environmental applications, particularly in extremophiles, which frequently reveal metabolic capabilities and evolutionary solutions not witnessed elsewhere in the microbial world (Coker et al. 2016). However, it is rarely possible to directly link the presence of a specific microbial taxon to a particular ecological process. Other methodological challenges include establishing the relative importance of biotic and abiotic factors in microbial ecosystem function, and determining the appropriate spatial and temporal scale necessary to discriminate links between microbiota and their ecological functions (Bissett *et al.*, 2013). Concurrently, a deeper understanding is required of human-induced impacts on the global microbiome through urbanisation, habitat degradation, climate change, and the introduction of invasive species, amongst others.

30~~25~~. How do we successfully establish microbial communities used in bioremediation?

31~~26~~. How important is the rare microbiome in ecosystem function, and how does this change with stochastic events?

32~~27~~. To what extent is microbial community diversity and function resilient to short- and long-term perturbations?

33~~28~~. What is the importance of spatial and temporal variation in microbial community structure and function to key environmental processes and geochemical cycles?

34~~49~~. How can we accurately measure microbial biomass in a reproducible manner?

35~~29~~. Which mechanisms do extremophiles use for survival and how can they be exploited?

Functional Diversity

Ecologists are increasingly turning their attention to classifying species based on their activity (function) within an ecosystem, rather than their genotype (Crowther *et al.,* 2014). This is particularly relevant for microbial ecology, in which species are hard to define, horizontal gene transfer is rife, and taxonomy is often blurred. Understanding how membership within complex and dynamic microbial communities relates to the function of that community is one of the key challenges facing microbial ecology (Widder *et al.*, 2016). This is true across a vast range of spatial scales, from microbial dyads to the gut of a *Drosophila* fly*,* to ancient trees and their associated ecosystems, right through to global biogeochemical processes. There is an urgent need to understand how the genome ~~or hologenome~~of ~~of~~ a microbial community (and in some cases, its host) relates to metabolic capacities. Conversely, there is also a need to understand how ecosystems depend on a particular organism or group of organisms for any given process and function. This section describes the need to move from simply describing microbial diversity to understanding what these organisms are doing, how they are doing it, and what biotic and abiotic drivers are controlling their activity. Each question may derive a suite of different answers, depending on the group of organisms, the habitat and the process.

36~~0~~. What are the mechanisms driving microbial community structure and function, and are these conserved across ecosystems?

37. What is the relative importance of stochastic vs. determinative processes in microbial community assembly?

38~~1~~. How conserved are microbial functions across different spatial and temporal scales?

39~~2~~. What is the relative importance of individual ‘species’ for the functioning of microbial communities?

40~~33~~. How much functional redundancy is there in microbial communities, and how does functional redundancy affect measures of diversity and niche overlap?

41~~34~~. How often are functional traits of microbes successfully conferred through horizontal gene transfer?

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

43. How do we move beyond correlation to develop predictive models that advance our understanding of microbial community function and dynamics?”

44~~50~~. How useful are synthetic communities for testing~~inferring~~ theories about microbial community dynamics and function?

Evolutionary Processes

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

45~~35~~. How can a bacterial 'species' be defined?

46~~36~~. To what extent is faunal and floral biodiversity influenced by microbial communities?

47~~37~~. To what extent do microbial communities have an equivalent to keystone ‘species’?

48~~38~~. Does the structure of microbial communities conform to the same ecological rules/principles as in other types of communities?

4~~3~~9. How do fundamental shifts in environmental conditions impact the trajectory of microbial evolution?

50~~40~~. What are the relative selective forces favouring microbial genome expansion or reduction?

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

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~~*Methods in Microbial Ecology*~~

~~Methods for assessing microbial diversity and community function have rapidly advanced in recent years, with a major shift from culture-dependent to molecular-based techniques that produce vast quantities of data (Rohwer, 2007; Biteen *et al*., 2015). Advances in technologies for the analysis of (meta)genomes, (meta)transcriptomes, (meta)proteomes and metabolomes, with associated computational biology tools, have revolutionised our understanding of microbial diversity and function, with multi-'omics' approaches providing unprecedented opportunities to assess genomic potential, gene regulation, expression and functionality *in situ*. There are still many challenges relating to methods for analysing and describing microbiomes, elucidating the roles these microorganisms play both individually and as a community, and how this relates to wider organismal function and environmental processes (Robinson *et al*., 2010). Amplicon studies also raise the problem of how to link taxonomic identity to functional ability. The vast datasets produced by the 'omics' technologies present unique statistical challenges, requiring new analytical techniques and approaches (Weiss *et al.,* 2016ab). Simultaneously, high-throughput culture-based methods are being re-invented for applications such as antibiotic discovery (Ling *et al.,* 2015; Oberhardt *et al.,* 2015). There is no one-size-fits-all method for a given type of study, but it is desirable to have a suite of robust methods that can be applied in a comparable manner to achieve results with a high level of confidence (Bustin *et al.,* 2009). As contemporary technological advancements improve the accessibility, throughput, resolution and cost of microbiome analysis, this section explores some of the new challenges that arise due to rapid advancements within the field, and other research questions that can be addressed.~~

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

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

~~44. How can we develop a standardized best-practice method for analysing sequence data to estimate relative abundance?~~

~~45. What is the most appropriate taxonomic level at which to compare microbial community compositions?~~

~~46. How can systems approaches improve our understanding of host-microbe interactions?~~

~~47. How do we move beyond correlations to determine cause and effect in microbial communities/ecosystems?~~

~~48. How can we better track the source and dispersal of particular microorganisms in real time?~~

~~49. How can we accurately measure microbial biomass in a reproducible manner?~~

~~50. How useful are synthetic communities for inferring theories about microbial community dynamics?~~

Society and Policy

We need to find ways to apply fundamental biological research to the benefit of society and policy. For example, collaboration with social scientists is crucial when investigating public understanding of microbial ecology, as well as using citizen science approaches to tackle microbial ecology research questions. Many questions relating to this area were discussed at the workshop, and here we present four additional questions that were developed at the meeting that relate to societal and policy-based aspects of microbial ecology.

- How can we best address supply and demand of information about microbial ecology between researchers, clinicians, policy makers and practitioners?
- How can we best use social and traditional mass media for early identification of emerging threats to animal and plant health?
- How can we develop an open access data repository or integrate existing databases to create 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 to the use of microbes in bioremediation and bioaugmentation?

Discussion

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

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

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

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

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

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~~Probiotics were discussed as a viable and promising alternative (Martín et al., 2013; Newaj-Fyzul et al., 2014; Smith, 2014; Fox, 2015), not only to improve individual health, but also to decrease disease susceptibility, enhance nutritional quality of food, and to mitigate the negative impacts of antibiotic use across humans, livestock, aquaculture and agriculture(Martín et al., 2013; Newaj-Fyzul et al., 2014; Smith, 2014; Fox, 2015). Developing personalized probiotic-based therapies requires complementary diversity and functional-based studies in order to elucidate the specific roles of microbiota in health and disease, and thus how microbial communities can be manipulated.~~

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

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

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

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

~~Recent major technological and analytical advancements have made previously inaccessible~~

~~taxa and ecosystems amenable to study. Despite the opportunities afforded by big data however, there are huge challenges when handling next-generation sequencing data, both in the size of the datasets and the spurious inter-correlation (compositionality) inherent to the sequencing process (Weiss *et al.*, 2016a). There is community-wide recognition that current methods of analysis are not adequate to deal with the big data produced by next-generation sequencing, and a number of groups have already attempted to establish standardised analysis methods (*e.g.* amplicon sequencing of bacterial and fungal communities (Thomas *et al.*, 2012; Smith & Peay, 2014), and bioinformatics of targeted and shotgun metagenomics (Bokulich *et al.*, 2013; McMurdie & Holmes, 2014; Oulas *et al.*, 2015, Randle-Boggis *et al.*, 2016). These methods now require validation under different contexts to keep analyses with similar aims and methods comparable (Weiss *et al.*, 2016a,b). This is no easy task given the plethora of decisions made throughout research design, from sample collection and storage to lab work and data analysis (Callahan *et al.*, 2016), each of which introduce complexity to a study's approach.~~

The need for open access databases and repositories, both in the context of data sharing as well as for methods and protocols, was reflected in the questions shortlisted for the *'Society and Policy'* theme. Discussions included the benefits of forming collaborative and open research communities, and the need to ensure the legacy of academic research through improving regulation and policy and engagement with the public. Fear-based regulation of research, grounded in alarmist or populist campaigns, as opposed to risk-based regulation built upon evidence, was identified as a possible obstacle to progress, which could be addressed through greater interaction between microbial ecologists and the public at both governmental and grass roots levels. Large scale assessments of ecosystem services and degradation acknowledge the paucity of data on microbial impacts, presumably because there are no convincing large-scale messages that can be derived at this stage (Norris *et al.*, 2011). Microbial diversity is therefore rarely considered when estimates of biodiversity are required for policy or management decisions. That said, ~~T~~the increasing recognition of the fundamental impact of the microbial world on the functioning of larger-scale processes has made the deliberate manipulation of the microbial world a controversial subject, which was reflected in the number of draft questions submitted related to bioremediation and bioaugmentation (see Supplementary Information). Collaboration with social scientists was identified as crucial in gauging 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 tools to tackle key microbial ecology research questions.

The 50 questions identified here cover a broad range of topics, but some over-arching themes recur across multiple questions, including a recognition that microbes play an important role in a variety of different processes and systems, which may be harnessed to solve real-world problems. There were some similarities between the questions identified here and those identified by previous workshops of a similar nature. For example, questions relating to soil health and biodiversity (Dicks *et al.* 2013), a requirement for developing a theoretical understanding of micro- and macro- ecological concepts (Prosser et al. 2007, Sutherland et al. 2013a) and disease dynamics (Prosser et al. 2007, Sutherland et al. 2013a) have a degree of commonality with this list. This indicates that the ecological theory underpinning many research questions transcends scientific disciplines, and that there is still much work to be done at both theoretical and applied levels. Within these 50 questions, we have tried to provide a focus for researchers addressing scientific questions from a microbial perspective, regardless of their background. It is expected that these questions will facilitate interesting discussion and new, exciting, interdisciplinary research. The list is by no means exhaustive, and we recognise that the questions presented here are relatively community-centric, primarily due to the recent expansion in methodological approaches that have improved our understanding of microbial community diversity and function. That said, other areas of microbial ecology should not be ignored or forgotten. ~~and g~~Given the rapidly evolving field of microbial ecology, it is expected that future workshops with a wide draw will be held to ensure that the identification of research priorities and areas of interest is a continuing process.

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Are all families of bacteria capable of acquiring pathogenicity or are there barriers to the exchange of cer

Are all functional traits horizontally transmissible?

Are comparisons of microbial community composition at phylum level overused and uninformative?

Are faunal and floral biodiversity driven by microbial communities?

Are outdated species and subspecies definitions impeding our understanding of microbial ecosystems?

Are prosperous and diverse microbial communities that can auto-regulate less harmful, or risk-prone, tha

Are social scientists and economists going to be present in the panels on animal and plant health of the E

Are species just a handful of relatively stable lineages within gene pools in which horizontal gene transfer

Are studies which consider microbes by genus adequate in light of observed interspecies variation?

Are synthetic communities useful in inferring theories about microbial community dynamics?

Are the genes related with metabolic function vertically or horizontally inherited

Are there any unexplored 'microbial frontiers'?

Are there difference in microbiomes of heather under different management regimes?

Are there differences between skin microbiome of rural compared to urban amphibians?

Are there species or just highly integrated microbial communities?

As climate change continues to impact the global ecology, can monitoring microbial communities give an

As climate change effects soils including salinity, water levels and temperature, will this impact on food p

As extreme environments are being perceived as offering the best option for novel antimicrobials what h

As fracking looks increasingly likely to happen in the UK, what will be the effect on the surrounding micro

As it is may already have gone past the tipping point for climate change, can evolution be monitored in e

At what spatial scale do we need to study microbial communities to answer applied or globally important

Can a better understanding of microbial ecology give us clues on how complex larger ecosystems functio

Can bacteriophages be used to eliminate certain bacteria in an environmental sample?

Can bioinnoculation based bioremediation techniques be deployed more successfully as a result of omics

Can endohyphal bacteria positively impact the environment through their host?

Can host community assemblages be used to predict parasite (micro and macro) community composition

Can media monitoring be used for early identification of new emerging threats to animal and plant healt

Can metagenome analysis completely replace phylogenetic markers to describe diversity in microbiomes

Can micro-organisms be cultivated directly in soils using greenhouses?

Can microbes 'prime' each other to degrade organic matter?

Can microbes go extinct?

Can microbial communities ever be considered stable or is stability an artefact of the spatial scale by whi

Can microbial ecology provide an alternative to antibiotics?

Can microbial ecology tell us anything about non-microbial ecology?

Can probiotic microbiota mitigate water-stress induced plant disease?

Can regional outbreaks of exotic tree diseases become opportunities for nature conservation?

Can the gut mirobione be manipulated to enhance health?

Can understanding the relationship between ash die back disease and microbial community be used in sp

Can we come up with a standardized method for estimating relative abundance of high-throughput data

Can we distinguish individual from interactive microbial effects? e.g. microbes that "turn on" (or off) path

Can we effectively synthesize microbial communities specialized in decomposing waste and recycling for

Can we genetically engineer endohyphal bacteria that are known to be the cause of plant disease to exer

Can we integrate our understanding of different microbiomes?

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

How can we examine ecological processes (e.g. N mineralisation) on a scale relevant to microbes?
How can we get young people interested in invisible, boring microbes?
How can we identify microbial species in the environment in real-time?
How can we improve the proportion of microbial diversity that is isolated and held in pure culture?
How can we interpret Koch's postulates in relation to polymicrobial infections as revealed by microbiome
How can we make microbial ecology relevant to policy makers?
How can we measure the hidden diversity? Overcoming arbitrary cut off values in next-generation seque
How can we prove that microbes have ecosystem-scale impacts?
How can we relate large-scale taxonomic information to fine-scale function?
How can we solve the most pressing career issues affecting early stage researchers in microbial ecology?
How can we stop pathogens from forming biofilms?
How certain is genetic decay in a symbiont
How cosmopolitan are microorganisms?
How diverse is a 'community'?
How do differences in microbial communities in human and animal microbiomes relate to different disea
How do gut bacterial communities interact with viral infections? Can we classify communities as "neutral
How do microbial communities interact to effect the phenotype of the host?
How do plant endophytes enter, become active in, and influence their hosts?
How do protists influence fungi in soil?
How do skin microbiota and UV interact in amphibians, especially at high altitude?
How do soil bacteria, viruses and archaea interact?
How do virus influence microbial ecology?
How do we establish effective sample sizes for studies of poorly characterised microorganism?
How do we sample environmental variables at an appropriate scale that are proximate to microbial comr
How does individual microbiota affect others at a community level
How does the environment select?
How does the microbiome influence health?
How important is the "rare" microbiome in ecosystem function?
How important is the microbiota in mediating adaptation that results in species invasions?
How is best to study the changing resource environment of microbial communities?
How is climate change going to affect the microbial communities in the drinking water distribution syster
How much functional redundancy is there in microbes relative to higher taxa?
How much functional redundancy is there in microbial communities, and how does that interact with dive
How much functional redundancy is there in the host microbiome?
How much intra- vs inter-specific trait variation is there?
How much microbial diversity in the soil has been lost through monoculture and what is the importance
How problematic is PCR amplification bias?
How similar are is the rhizosphere biodiversity of microbes from the same species of tree located in differ
How stable are microbial communities, and how can we assess their stability, particularly in changing env
How to foster interdisciplinary approaches in and around microbial ecology?
How to solve the leaky pipeline of female microbial ecologists in academia?
How to solve the peer reviewer crisis in microbial ecology?
How well do current species distribution models developed with microbial systems predict the distributic

How will different temperature and humidity regimes reflecting likely climate change scenarios affect lea
How will predicted climate changes influence the free-living stages of terrestrial and aquatic helminths?
Intensive farming may involve high levels of broad spectrum antibiotic usage. This in turn will end up in t
Is Alan McCarthy the oldest microbial ecologist on the planet?
Is everything everywhere?
Is it necessary/important to document microbial diversity? Should it be done at the genus/species level?
Is it still relevant to dig for antibiotics?
Is it syntrophy the main metabolic mechanism sustaining microbial cooperation
Is it time to stop cataloguing bacterial community compositions in different habitats and put a bit more e
Is microbial abundance and/or diversity driving ecosystem functioning?
Is microbial ecology restricted by the red tape surrounding releasing microbes into the environment?
Is the carbon source used in general/selective media the limiting factor for some un-culturable micro-org
Is the growth in microbial ecology publication sustainable?
Is there a relationship between microbial community and ecosystem functioning, and is it observed acros
Is there a way to overcome big data?
Is there any point in doing comparative metagenomics?
Is there going to be an improvement in the file-drawer problem (i.e. the tendency of positive results to b
Is there such thing as a microbial pathogen?
Is virus burden and shedding intensity in mammals amplified when co-infected by helminths?
Many hands make light work? What is the role of functional redundancy in ecosystem processes?
mechanisms extremophiles utilise for survival and potential uses elsewhere
No biome is an island. As interest in this area increases, how do microbiomes interact outside the microb
Not Waving but Drowning; how much evidence do we need to fully interpret omics data?
One size fits all? Is there a relationship between microbial and "macrobial" ecology?
Pathogens get a lot of mainstream attention. Should researchers do more to promote positive microbial
plant host interactions, symbiotic relationships
Rapid detection and identification of microbes
Sequencing data has increased significantly in the last 15 years, will bioinformatics be the future of our st
Should new species descriptions based entirely on sequences be allowed?
Should researchers place greater emphasis on evaluating microbe functional traits (applied aspects), and
Should we be concerned about the extinction of microbial species?
Should we couple microbial ecology with Invasive Non-Native Species (INNS) risk assessments?
Society and policy makers; do they value microbial ecosystem services?
Soil microbial communities and the role they play
Soil microbial communities and the role they play
Soil profiling has been done for many years, can we estimate the type of organisms in a soil using only inf
Taxonomy vs function: Do functional groups exist and in a world of horizontal gene transfer. Do 16S sequ
The rare biosphere; waiting in the wings or ghosts in the machine?
There are many factors that alter and select which organisms belong in a specific environment, througho
To what extent are microbial species distributions influenced by climate? what would the consequences
To what extent do microbial communities return to their "original" state after short-term perturbations?
To what extent does microbial community composition reflect functional redundancy in a habitat or ecos
To what extent is among human or animal variability in microbiome related to evolutionary processes oc

To what extent is animal health and welfare influenced by their microbiome and does this have the poter
Universal sequence primers - can they ever exist?
What are the biggest gains in microbial ecology of the last decade and what are their implications for the
What are the consequences of antibiotic use in microbial communities in freshwater and soil environmer
What are the environmental hazards associated with bioremediation and biocontrol, and what can we dc
What are the likely impacts of climate change on plant diseases in understudied regions such as Africa, th
What are the links between microbial genomics and metabolomics?
What are the main biotic and abiotic determinants of population structure in microbial communities in w
What are the main mechanisms by which nutrients accumulated by saprotrophs are released to the envir
What are the major constraints on microbial life on land and water, and how do they differ?
What are the mechanisms by which the gut microbiota protects its host from pathogens?
What are the mechanisms used by mycoviruses to confer host fitness to its environment?
What are the microbial ecology consequences of the cultivation of exotic tree species?
What are the most successful applications of microbial ecology in the real world?
What are the opportunities to determine cause or effect relationships in microbiome studies?
What are the primary mechanisms that mediate microbial interactions within a host? and the strength of
What are the survival implications of skin and gut microbiomes of captive bred or head-started animals?
What are the traits of microbial species that are a) globally ubiquitous b) endemic to certain areas?
What can animal, human and plant health policy-makers learn from evolutionary ecology?
What effect will rising water levels have on coastal microbes and their diversity?
What is the 'functioning' of a microbial community?
What is the acceptable amount of disease in a healthy forest ecosystem?
What is the actual relevance of Black Queen Hypothesis
What is the average carbon footprint of a paper in microbial ecology and how can it be reduced?
What is the best method to assess the contribution of rare vs abundant species in a microbial community
What is the best strategy to control and stop waterborne outbreaks?
What is the best way of monitoring drinking water safety?
What is the ecological meaning of an OTU?
What is the ecological relevance of the internalization of bacterial pathogens by protozoa in terms of the
What is the importance of biotic vs abiotic characteristics in determining microbial community compositi
What is the importance of photosynthetic symbionts of larval amphibians?
What is the relative importance of inter- and intra-specific diversity in fungal communities?
What is the relative importance of stochastic vs. determinative processes in fungal community assembly
What is the relative role of competition versus cooperation for microbial biodiversity
What is the role of bacteriophages in biofilm dynamics in natural environments?
What is the role of the microbiota in speciation processes?
What is the true link between diversity and function in an ecosystem?
What mechanisms are distinct in microbial ecology as opposed to conventional ecology?
What methodological processes do we need to develop to give a holistic view of microbial diversity and f
What methods can we use to marry microbial diversity with function, what role could proteomics play?
What new sequencing techniques do we need to improve our understanding of microbial ecology?
What new technologies are available to detect cross-feeders in an environmental sample?
What proportion of fungi carry endobacteria, and what effect does this have?

What scales are appropriate for studying microbes?
What selection pressures does the modern world place on evolving microbes?
What technologies are needed to advance microbial ecology?
What's the best way to manipulate the microflora?
Which factors determine the host range of microbial pathogens?
Which factors determine the structure of gut microbiomes?
Which factors determine whether Wolbachia increases or decreases its host's susceptibility to pathogens
Which factors trigger 'covert' infections to become 'overt', impacting host health?
Which factors trigger the emergence of new microbial pathogens in humans and wildlife?
Which is the most effective way of disinfecting drinking water?
Why are some microbial pathogens often 'covert'?
Why do genomes contain redundant copies of genes
Will 16S rRNA be supplanted as the key phylogenetic identifier
Will fungal underrepresentation in the life sciences keep on decreasing as was the case over the last two
Will metagenomics ever supplant next generation sequencing?
Will metaproteomics ever become an important major methodology in microbial ecology?
Will microbes become commonly used as biological control agents of invasive alien plants in Europe?
Will ocean acidification or temperature increase provide the greatest selective pressure for microbial ma
Will the challenge of fungal genomics to the dogma of name-based biosecurity be taken on board by phy
Will the culturing of the other 99% of bacteria be possible with increasing understanding of syntrophy an
Will the flood of new papers on climate change and microbial ecology lead to increased specialization an
Will the use of network theory in plant epidemiology help prevent plant health emergencies such as Eur
Will there be substantial funding for research and management of tree diseases over the next decades, o
Will there ever be a cohesive species concept which applies to bacteria?
Will there ever be a unified V 16S rRNA region set of primers to capture total diversity
Will we be able to find new antibiotics in microbial species? Should we prioritise funding for this?
Will we ever be able to differentially monitor infective and non infective viruses in the environment?
With specific reference to non-model organisms. We know metazoa harbour a diverse array of microbial
With the amount of -omics sequence information coming out every week, how can scientists is it worth t

tain genetic elements which are required?

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

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

based techniques?

n?
n?
?

ch the community is investigated?

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

diation?

apacities, genomes)?

ane, etc)? What are the implications of microbial species loss in these cycles?

volution?

microbial communities or do this methods get bias from collecting too much material from dead

oot, size of the whole tree, maturity of the tree?

nunity in streams?

r microbiota?

re presumably barophilic?

n experimental artifacts?

eld?

making bodies

sity in soil microbial ecosystems?

se â€ ˜big dataâ€™ projects?

studies?

ncing.

se conditions?

ising" and "enhancing"?

nunities 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)?

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

f microbiota of umbrella species?

he faecal matter of livestock and deposit on the land. What will be the long term effect on micro

ffort into virus and microeukaryote ecology?

anisms?

s ecosystems (e.g. marine, freshwater, terrestrial, gut microbiota)?

e more easily published than negative ones) in microbial ecology?

iome environment?

effects?

udies of microbial ecology?

less on documenting diversity?

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?

curring within an individual's microbiome?

ntial to affect conservation efforts?

future of the field?
nts and how they influence human health?
to prevent them?
e Middle East and Central America?

ater distribution systems?
ronment?

evidence for them?

?

ir survival and spread?
on, and how does that influence ecosystem functioning?

unction?

?

decades?

rine organisms?
tosanitary regulators?
d the rise of metabolomic and transcriptomic studies
d communication problems within the field?
pean ash dieback?
r is the recent surge in the UK a short-lived burst?

diversity. How can changes in microbial communities, either through changes in community com
he while for scientists to create alliances so this information can be quickly available for other sci

cells?

?

bial population as a driver for antibiotic resistance?

which are the new most important factors that are of interest to microbial ecologists?

position or microbial evolution, help long lived (annual +) animals adapt to rapidly changing envir
entists that might be interested in the same information? Joining forces throughout societies.

onments?