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 eight themes: Host-Microbiome Interactions; Health and Infectious Diseases; Food Security and Human Health; 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) *Food Security and Human Health;*
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 (‘*Food Security and Human Health’*).

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?

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. 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 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. 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 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 application, 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 or hologenome 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 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. 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*. 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).

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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