Prosser James (Orcid ID: 0000-0003-1757-5102)

How and why in microbial ecology: an appeal for scientific aims, questions, hypotheses and theories

James I Prosser

Institute of Biological Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen, Scotland, AB24 3UU, UK

Correspondence author: James Prosser, 1 Hillhead of Mosstown, Udny, Ellon, Aberdeenshire, AB41 6QA, United Kingdom

Email: j.prosser@abdn.ac.uk; Telephone: +447802 959364

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1462-2920.16221

This article is protected by copyright. All rights reserved.

Accepted Article

Originality Significance Statement

This invited opinion article discusses the need for, and properties of good, important and meaningful questions and hypotheses in microbial ecology research. In doing so, it reviews relevant aspects of the philosophy of science and the processes adopted in studies of microbial ecology. The opinions expressed are my own, some of which have been published in Prosser (2020), where the emphasis of was on induction, inference and hypothetical deductivism, rather than detailed analysis of scientific questions and hypotheses. There is therefore some, but not significant overlap in material and the two articles serve different functions. It is hoped that this article will significantly influence approaches to research in microbial ecology, increasing emphasis on scientific aims, questions and hypotheses.

Accepted Article

Summary

This article precedes a series of articles on the important questions, hypotheses and theories in microbial ecology. It considers why, as scientists, we ask questions and propose hypotheses and what makes them important, good or significant. Emphasis is placed on 'scientific' questions, the need for scientific aims and on possible reasons for, and inadequacy of aim-less studies and question-free. Current global issues surrounding the climate crisis, pandemics and antibiotic resistance focus attention on science and scientists. They exemplify the urgent need for greater understanding of the interactions between microbes and their biological and physicochemical environments, i.e. of microbial ecology. They also provide examples of reaction against science and scientists and highlight why we must be clear what defines (good) science, its power and limitations, and ensure that this is communicated to stakeholders and the general public.

Personal note

I was first forced to think about the nature of science as a microbiology undergraduate sharing a flat with a physics undergraduate. He, and his friends, patiently and sympathetically explained how physicists look up to mathematicians but look down on chemists, who look down on biologists, looking down on social scientists, economists, etc. The emphasis on quantitative microbiology within my degree course induced some forbearance and tolerance, but it was clear where microbial ecology fell on the 'hard-soft' science spectrum. They also attended lectures on the philosophy of science and introduced me to the ideas of Karl Popper, who they admired, and Thomas Kuhn and to the sociology of science. Despite the inferiority complex generated by this assault, I studied for a PhD, and my interest in these topics grew: if I was to become a Doctor of Philosophy, maybe I should know where philosophy fitted in. I was influenced by Popper's ideas, was interested in those of Kuhn, but found books on the sociology of science difficult to penetrate. Popper and Kuhn illustrate two approaches:

prescriptive, in which philosophers highlight deficiencies in different approaches and suggest solutions, and descriptive, in which the behaviour of scientists is described and analysed.

These readings influenced my research and, if I had any influence, that of my research group. The philosophy of science has, of course, developed in the intervening 50 years and provides valuable reading, even though links between scientists and philosophers are not always strong. They show how other scientific disciplines work and challenge many issues and assumptions that we take for granted at our peril. For example, as practising scientists, we may lack appreciation of ideas on causality, logic or reason, or how concepts act as a lens between our observations and the real world, and we may not be sufficiently critical of accepted techniques and background knowledge or the rationale behind different approaches to science. They also consider what differentiates science from pseudoscience, superstition and belief. (See Chalmers, 2013; Craver and Darden, 2013; and The Stanford Encyclopedia of Philosophy for good introductory material on these and other topics discussed below.)

What is science and the scientific process?

The aim of science is to increase our understanding of how the world works. This process begins with identification of a problem or deficiency in our understanding and establishment of a scientific question. The science of microbial ecology, therefore, aims to understand the ways in which microbes interact with their natural environments: to understand and explain observed phenomena, to predict if and how they will recur in the future and, potentially, to control them. None of us lives in a vacuum and it is difficult to imagine a phenomenon in microbial ecology about which we would claim complete ignorance and an absence of background knowledge. We are therefore likely to have some understanding, and the aim is usually phrased as 'to increase our understanding of…', i.e. we observe something about microbes and their environments that we cannot explain or fully explain and attempt to explain it. The scientific process therefore involves several stages:

Accepted Article

1. Observation and characterisation of a phenomenon.
2. Assessment of the ability of current knowledge to explain the phenomenon or the need for new explanations, i.e. hypothesis construction, and generation of predictions.
3. Hypothesis-testing, comparing predictions with independent observations, followed by rejection or revision of hypotheses and further testing.

Observation and characterisation of a phenomenon – posing the question

Initially we must identify and characterise the phenomenon of interest and establish the scientific question. This process is crucial, as it determines the breadth, scope, feasibility and significance of our study and involves a number of considerations. (For convenience, I will illustrate these with an example from my own research group: the phenomenon of nitrification (by ammonia and nitrite oxidisers) in acid soils; see Prosser & Nicol, 2012 for a review.)

- The reliability of the observations describing the phenomenon should be confirmed. Has it been observed once, or repeatedly and reproducibly, and were observations performed well? This may lead to further observations to confirm the phenomenon. For my example, nitrification had been reported in a large number of acid soils.
- Why is this a phenomenon? What specifically do we not understand, or only partially understand? This should be clarified to ensure that questions are well-characterised and well-formulated, rather than vague. Many publications review a research area and conclude that we lack understanding, without stating precisely what is not understood, why it is important that it should be understood and how their study might increase understanding. The question that we pose highlights, delineates and characterises our lack of understanding. Precise and careful formulation of the question is therefore important. For my question: nitrification in acid soils was a phenomenon, and difficult to understand, because available cultures of ammonia oxidisers could not grow in liquid culture below pH 7.

Accepted Article

- Importantly, scientific questions are 'how' and 'why' questions, rather than 'who is there' and 'what are they doing'. If we can answer such questions, by considering mechanisms, then we begin to understand and explain what has happened and predict what might happen in the future. We were asking 'how' does ammonia oxidation occur at low pH in soil.
- Are observations qualitative or quantitative. Qualitative questions, e.g. presence/absence, more/less, are much easier to answer but may be insufficient; quantitative questions are more ambitious and require greater understanding. Our basic question was qualitative, but became more quantitative, as we sought to explain why rates of nitrification appeared to be independent of pH, requiring much more information on metabolic rates and environmental characteristics.
- The phenomenon should obviously be of interest to the researcher, driven by curiosity, and intellectually challenging, if only to ensure enthusiasm and motivation. We are each curious about different things and, in that respect, 'interest' may be subjective. We were interested in nitrification, but realise that other researchers have other interests.
- How universal or general is the phenomenon and question. Our question concerned ammonia oxidisers and soil, and in that sense might be seen as narrow. However, many other microbes are active in acid soils, other acidic environments exist, and our answers may be generally applicable. Formulating the question as a universal question certainly increases its significance.
- The 'so what' question should be considered. If we discover how nitrification occurs in acid soils, what difference will this make? For us, it will explain an interesting phenomenon and an important aspect of nitrifier ecology. If the answer is universal, it will fulfil the same function for all microbes, in all acidic environments.
- In addition to advancing fundamental aspects of microbial ecology, does the question have applied relevance and interest to industry, policy makers, environmental agencies, etc? Will

Accepted Article

these stakeholders be satisfied with qualitative answers? Answering our question will be important, as nitrification leads to considerable fertiliser loss from acid soils, globally, and is a major source of nitrous oxide emissions. For those with interests in these applied aspects, quantitative information would be extremely valuable.

Choosing and identifying the question is the most important part of a scientific project. It is therefore justifiable, if not essential, to spend considerable thought, time and discussion getting this right, to avoid wasting time trying to answer bad, superficial, vague or trivial questions.

Answering the question – hypothesis construction

We must now try to explain the phenomenon and answer the question. The explanation takes the form of a hypothesis or theory or a law. Although not consistently or precisely defined, these terms imply increasing confidence. Hypotheses usually refer to explanations for which there is little or no empirical evidence; theories imply greater confidence, following experimentation, and laws imply broad acceptance (but see next section). To quote Popper (1959), 'Theories are nets cast to catch what we call 'the world': to rationalise, to explain, and to master it. We endeavour to make the mesh ever finer and finer.' This nicely reflects the function and nature of theories in the scientific process; he also equates hypotheses with myths, highlighting the fact that, until tested experimentally, they have limited scientific value.

Initially, we attempt to explain the phenomenon using background information and existing ideas and hypotheses. If successful, we may move on to another question or we may decide to test an existing hypothesis in our system. An important feature of science is the continual challenging and critical testing of existing theories. If not successful, we may need to modify existing explanations or hypotheses or develop new hypotheses. These must conform with existing data, but will require something new and will require thought. This process of hypothesis construction is to be embraced and not feared, and is the job of every scientist. It is

Accepted Article

as important as experimental work and not something to be left to others. For my example, there were several potential explanations for nitrification in acid soils, including: protection from effects of low pH through surface growth; existence of alkaline microenvironments; acidophilic ammonia oxidisers, heterotrophic nitrifiers and pH-independent urea hydrolysis.

In constructing hypotheses, we need to consider a number of criteria:

- A hypothesis should explain all existing observations, or provide a very good reason for excluding or rejecting observations that are not explained.
- It should be based on mechanisms, i.e. providing information on how or why the observed phenomenon occurs.
- The hypothesis, and the assumptions on which it is based, should be clearly and precisely defined and simplifying assumptions should be discriminated from those defining the proposed mechanisms(s). If we suggest that surface attachment enables ammonia oxidation at low pH, a simplifying assumption might be that this applies to all ammonia oxidiser phylotypes, thereby 'isolating' this explanation from others. This does not eliminate alternative explanations, including the existence of acidophilic phylotypes, and does not, invalidate the hypothesis, any more than seeing a helicopter take off invalidates the law of gravity. The phenomenon may result from combined effects of different explanations, requiring a more complex hypothesis.
- The hypothesis should be critically testable and, in particular, should be rejectable or refutable. This is partly for practical reasons. For example, we cannot prove an absence, but only lack of detection), so inability to isolate an acidophilic strain could not lead to rejection of this hypothesis. Initially, reliable techniques were not available for assessment of community structure and we could not test selection for acidophilic strains in acid soils. Other reasons for focusing on rejection and refutation are discussed in the next section.

Accepted Article

- The hypothesis should be novel and risky, the latter increasing testability. Proposing acidophilic strains in the absence of a cultivated isolate is risky.
- Alternative hypotheses need to be capable of generating predictions that discriminate them.
- Better hypotheses have greater 'content', e.g. explanations for activity of all microbes, rather than ammonia oxidisers only, in acid soils will require more information.
- The hypothesis should be quantitative, rather than qualitative. Quantitative hypotheses have much greater content and can be tested more critically. They are riskier. The inability to prove an absence in practice made our hypotheses quantitative, e.g. in suggesting that nitrification rates on surfaces will differ quantitatively from those of suspended cells.

This process is crucial in delimiting and circumscribing the scope, ambition and feasibility of a study. The hypothesis is based on the question and we need specific hypotheses to address specific questions. Hypothesis construction may well lead to modification of the question, maybe defining the question in a different way, making it more universal or specific. This process certainly made us think harder about nitrification in soil and more carefully about the precise questions we were asking.

Hypothesis testing, rejection, revision and further testing

Hypotheses must be tested by comparing predictions with data that are independent from those used in their construction. This effectively precludes the use of previously published data in hypothesis testing, as any hypothesis should explain data that have already been observed. The nature of new data and how they are obtained depend crucially on the hypotheses and underlying assumptions. Experimental systems must aim to ensure that any simplifying assumptions are obeyed as much as possible, allowing isolation and testing of the mechanism-based assumptions. Importantly, the hypothesis and assumptions determine the techniques, equipment and experimental systems to be used, and not the other way round. I am sure that I am not the only supervisor to be approached by a student with an experiment designed with the

Accepted Article

real intention of learning a new, trendy technique. My response is always to ask 'what's the question?' (such that one student suggested that this be written on my headstone when I was buried, with an implicit assumption that this should happen soon). The hypothesis tells us what needs to be measured and, importantly, what needs not be measured, all of which should only be considered once a clear hypothesis has been formulated. The complexity of microbial communities and their environments, and their interactions at microscopic scales make hypothesis-testing challenging, but should not be viewed negatively. This provides interest, intellectual challenge and potential for problem-solving, ingenuity and innovation that make science exciting.

Many hypotheses can be proposed to explain a phenomenon. (One philosophical argument suggests that an infinite number of potential hypotheses can explain any phenomenon, so the probability of one being correct is 0 and any hypothesis will eventually be disproved.) Practically, or pragmatically, the number of hypotheses is reduced, through our background knowledge and by rational consideration of which hypothesis(es), including any new ones, is/are most likely and testable. Similarly, there are an infinite number of data that will support any hypothesis. There is therefore a real danger of uncritically accumulating evidence for a hypothesis, leading to false confidence and the fatal attraction of absolute knowledge. Popper (1959, 1963) pointed out that we can never 'prove' a scientific theory, as there may always be an experiment that we have not yet thought of that will disprove it. He therefore proposed that critical testing of hypotheses requires designing experiments to falsify or refute our hypotheses. Understanding then increases by eliminating rejected explanations and retaining (but never fully accepting as truth) those which we cannot reject.

Designing experiments to provide only supportive data may be psychologically and emotionally easier, but is not good science. We may want our 'pet' theory to be supported, but we learn much more by finding a hypothesis is incorrect, as this allows us to eliminate false

Accepted Article

hypotheses and explanations. Remember, we can never accept a hypothesis or theory as correct. Eventually, all theories are shown to be incorrect and will be replaced by better theories. These new theories will be better because they explain previous observations, including those used to falsify earlier theories, but they will then, themselves, be subjected to critical testing.

For our example, experimental work indicated that surface attachment provided some protection from low pH but, alone, could not explain nitrification in pH 4.5 soils, allowing rejection of this hypothesis. Eventual isolation of an acidophilic strain capable of cultivation in liquid culture, meant we could not reject acidophilic strains as a potential explanation. Further attempts at rejection (e.g. detection of activity of similar strains in acid soil microcosms and high relative abundance of similar strains in other acid soils) also failed. We can never prove this explanation to be universal, without examining all acid soils on the planet. However, if nitrification is observed in a single acid soil with no detectable acidophilic ammonia oxidisers, we will need to reject it as a universal hypothesis. This will then require formulation of a new hypothesis, with greater content, whose subsequent testing will increase understanding further.

Rigid adherence to the process of conjecture (hypothesis construction) and refutation is difficult, as it presents the prospect of continually trying to disprove a hypothesis and never moving on to other questions. Popper, and others, developed the theory, introducing some pragmatism. For example, rejecting a theory outright may not always be necessary, if there are sound reasons for modifying it to agree with counter data, but further critical testing is then required. Nevertheless, the basic ideas apply: we can never prove a scientific theory, we will never have absolute knowledge, theories are not proved by accumulating supportive data and understanding increases by attempts to falsify critically testable hypotheses. We learn by our mistakes. Even if we fail to reject our hypothesis, we cannot accept it as the truth. We may need to revise and retest it or publish it as being corroborated (but not confirmed) by the data. If our hypothesis is rejected, we should not be disappointed, assuming that the hypothesis is

Accepted Article

Accepted Article

interesting and experimental testing well designed and reliable. Rejection of the hypothesis should be published with pride, as this will have advanced understanding.

A problem

Proposing, testing and revising hypotheses that increase understanding of observed phenomena is the basis of the 'scientific method'. It is how science grows and attempts to provide order to the world, enabling explanations and predictions. Unfortunately, we have a problem as this does not appear to be the most common approach in microbial ecology. Two personal experiences of my own illustrate this problem. The first was a 2-day discussion by 30 – 40 soil microbial ecologists of an already-funded project for extensive sequencing of 16S rRNA gene sequences in a soil. Discussion of sampling protocols, treatments, replication, extraction techniques, primers, databases, etc., was lively. Only late in the second day did someone ask 'What is the aim of the study?', i.e. what is the scientific basis for the project and its funding. There was no answer. Extensive and expensive measurements were being discussed without considering why they were going to be made, with no scientific aim or question. The second, a meeting on a microbial ecology topic, concluded with two discussion sessions, on methodology and key questions. The methodology session was highly active, with many contributions and proposals for new techniques that were considered essential for future progress. The session on key questions was met with complete silence, with an embarrassed chairman struggling to initiate discussion. Researchers therefore knew what techniques were required but not what they were required for. These are not exceptions. One analysis (Prosser, 2020) indicated that approximately 90% of papers in major microbial ecology journals had no scientific aim and did not address a scientific question (Prosser, 2020). These journals reflect the 'best' (most cited) microbial ecology research, and the picture would be worse if we considered studies rejected by these journals, appearing elsewhere or never published. Why is this and what attempts are made to justify non-scientific studies?

Aim-less studies

The word 'discovery' appears frequently in definitions and descriptions of science. It refers to the discovery of explanations or mechanisms: discovering how or why something happens. In microbial ecology, it has taken on a new, disturbing meaning, in which 'discovery-led' studies refer to searches for new sequences, organisms, genes, genomes, etc. This is reflected in the plethora of descriptive papers that uncover, discover, unravel, reveal, unveil, disentangle, unscramble and unearth microbial diversity. Undoubtably, molecular techniques have changed our view of microbial diversity in natural communities, addressing major technological challenges and requiring considerable imagination, ingenuity and skill. Unfortunately, merely describing what is present in an environment, i.e. who is there and what (potentially) they are doing, does not increase understanding. Criticism of these descriptive studies, particularly those of microbiomes, generates a number of responses:

- 'They provide data required for study of a new environment; how can you study a new environment without knowing what is there?'. This response begs a number of questions: why study the new environment? why is it important? what do you do not understand? what can you not predict? why might you want to predict this? is it likely to be different from previously studied environments? if so why and how? and why might these differences influence community composition or activity? We must presume, or at least hope, that a project would not be funded without posing such questions, answers to which immediately provide the basis for scientific questions that should drive the study. These questions invite analysis of published studies of similar environments or, if none is available, stimulate thoughts on which organisms might be expected. They focus attention and provoke thinking on which aspects of the ecosystem and which microbes might be important, whether and how microbiomes are likely to differ, etc. In other words, they provide the basis for specific scientific questions, highlight what is understood and gaps in understanding and direct

Accepted Article

experimental techniques and experimental design. It is always possible to begin study of a new environment with a scientific questions because you will always have some idea of what might be there on which to base testable hypotheses.

- 'They provide a baseline, reference, community against which we can assess changes or differences.' This is related to the idea of a 'typical' e.g. soil, ocean, sediment or gut community. Pragmatically, we now have sufficient database information on communities from a wide range of environments to predict what we might and might not expect. However, communities are dynamic and change, temporally and spatially. If temporal, spatial or treatment-type changes in communities are being studied, then the above process should be carried out, i.e. think about potential mechanisms through which communities might change or differ and use these as a basis for hypotheses and predictions that can be tested experimentally to address scientific questions.
- 'At worst, the data will generate information that might be useful to other scientists.' This implies that the researcher is not a scientist and has no scientific questions, but that others, who are scientists and do have scientific questions, may be able to answer their questions with the data. It begs the question: why not fund the scientists who have the questions, or at least ask them what sort of data they need? Critical testing of hypotheses determines what data are required, rather than hoping that relevant data have already been obtained and are residing, unloved, in a database. The complexity of microbial communities and their environments requires precise questions that require specific data, rather than vague questions and whatever data are in repositories. It is, of course, possible to find examples of, e.g., sequence data that have been used by others, but this will be a small minority of descriptive data that have been obtained and is very unlikely to have satisfied all of the requirements for hypothesis testing.
- Finally, 'we might get lucky and discover something new'. See 'serendipity', below.

Accepted Article

Science does not advance through aim-less observations. In practise, most observations are not aim-less or question-free, even survey or 'baseline' data. I suspect nobody would admit to making random observations, but many fail to think how descriptive studies can be transformed into genuine scientific studies, rather than merely expanding burgeoning repositories.

Studies based on weak or superficial questions

Surveys are obvious examples of descriptive studies with no questions. Others can be based on non-scientific, weak or superficial questions, with no potential for increasing understanding. Common examples are those that examine the effect of a particular factor on a process or community, comparisons and searches for correlations and patterns. Of course, each of these can be powerful tools when used to test hypotheses. Rather, I am referring to superficial, look-see 'experiments' performed in the absence of a question or hypothesis. These masquerade as scientific studies and often use the terms hypothesis and question, but the hypotheses are 'working hypotheses', with no mechanistic basis, and the questions are 'what if' questions, rather than 'how' or 'why'.

For example, we might ask the question 'what' would happen to a bacterial community 'if' fertiliser was added to soil and a working hypothesis might postulate that addition of fertiliser will change the community. The first thing to note with such 'effect of' studies, and equivalent comparison and correlation studies, is the absence of a phenomenon. They are not trying to explain anything; they are 'look-see' studies. Secondly, the working hypothesis has no mechanistic basis or explanatory power. If the community does change, understanding is not increased, because there is no phenomenon to understand; even if there was, if one was implicit, the hypothesis provides no explanation. However, as discussed above, such an experiment would not be proposed in ignorance of potential effects of fertiliser. Observations of community changes will be discussed at length, in the context of previously published data and background information on bacterial physiology and other potentially relevant factors.

Accepted Article

Accepted Article

However, no effort is made to use this same information, prior to experimentation, to generate hypotheses about specific effects of fertiliser addition. The experimental observations could then have been used to test predictions of these hypotheses. In fact, predictions of the hypothesis would inform experimental design, determining and delimiting how the experimental work should be performed and the data that were necessary, rather than attempting to measure everything.

Similarly, comparisons of communities or processes in different environments or after treatments, and correlations between community composition and collections of environmental factors are scientifically meaningless without prior, explicit consideration of why communities or processes might be different or why, and which, correlations might exist. Discussion sections of such studies will cite many publications that are consistent (usually), or not, with newly observed correlations, and may propose reasons (hypotheses) why, but few generate such hypotheses from (the same) publications prior to the study, such that the correlations could provide a test.

We can therefore summarise the benefits and limitations of aim-less descriptive and question-free studies:

Benefits:

- They are intellectually and often technically easy. They require no thought about mechanisms or hypotheses, merely observation. This can generate relief for a student and supervisor, but no scientific advance.
- If using a new technique, it would be surprising if no 'new' data were obtained. However, this applies equally if the technique is being used in a scientific study. We developed 16S rRNA-based techniques for soil ammonia oxidisers to test the hypothesis of selection for acidophilic phylotypes, but discovered and could not ignore very high and unexpected

diversity: a new phenomenon raising new scientific questions. The probability of obtaining novel findings when using new techniques is therefore just as great from question-driven studies as descriptive studies, while the probability of appreciating novelty is greater for the former.

- You may be lucky and something interesting may 'fall out' of the data, magically. (see serendipity below).

Limitations:

- Descriptive studies elicit and encourage the practice of 'making a story out of the data', a chilling phrase, especially given it implications, if unintentional, of fiction and fantasy. In other words, data are scoured for something interesting to facilitate publication. To an extent, the data provide answers, and generate feverish searches for appropriate questions. At worst, and fraudulently, this can lead to a hypothesis, derived from the data, being portrayed as the hypothesis that drove the study and the experimental programme. More commonly, 'inference to the best explanation' will involve attempts to cite work, chosen subjectively, that conform with findings and to dismiss those that disagree. This may result in hypothesis generation, but no testing.
- Publication of descriptive data legitimises this approach and encourages its adoption.
- Question- and hypothesis-independent studies can be considered as intellectually unambitious. As indicated above, there are always underlying reasons behind descriptive studies which can be used to develop scientific questions and hypotheses. It is essential and efficient to convert these into testable hypotheses; this is our job as scientists.
- Descriptive studies have no bounds or limits. We can always obtain more sequences, more rate measurements, analyse more samples, etc., and there will always be a newer and better technique on the horizon claiming to provide a better description.

Accepted Article

- In the absence of an aim, or a question demanding an answer, we will not know when sufficient data have been obtained. Consequently, descriptive studies are limited by resources, rather than scientific requirements, and tend to be judged on the basis of volume of data rather than their ability to address scientific questions.
- Some question-free studies declare an aim of increasing understanding, but describe no phenomenon and provide only vague statements about lack of understanding. We will never have full understanding or absolute knowledge. Stating that we lack understanding without identifying the specific 'lack' that is thought to be important, and why, is meaningless and makes the study meaningless.

Serendipity

While the scientific method outlined above is the best means at our disposal to advance microbial ecology, we cannot ignore the role of luck and good fortune. Aim-less and question-free studies rely on serendipity for any scientific value. However, as discussed above, recognition and awareness of the significance of discoveries are more likely by researchers already thinking deeply about important scientific questions, than those focussed only on descriptions. 'In the fields of observation chance only favours prepared minds' (Pasteur, 1854).

Elephants in the room

Many human characteristics are beneficial to scientific research, e.g. curiosity, observation, creativity, imagination, rational and critical thinking, scepticism, communication, etc. Other characteristics can influence the purity of our scientific ideals, e.g. selfishness, greed, egocentricity, pride, overambition, self-promotion, jealousy, over-competitiveness, etc. You will be able to add to this list and this is one topic for those studying the sociology of science. None of us is immune and I certainly cannot claim innocence of some of the sins highlighted above. Nevertheless, we should be vigilant and self-critical to reduce their influence on our

Accepted Article

science. Other factors are societal. A PhD student and their supervisor will be relieved to obtain data that provide something to analyse and discuss, regardless of its scientific importance. A researcher may (often justifiably) believe that their c.v., career, promotion prospects, salary, etc., depend more on the number of papers, journal impact factor and their expertise in an inventory of modern techniques than on their innovative ideas or scientific discoveries. Journals and their publishers, despite admirable statements in their Scope and Aims, will be influenced by a desire to fill issues and increase citations, impact factor and sales, placing pressure on editors and reviewers. Funding agencies, despite fine words, may be more influenced by trends, fashions, new technologies and government policies than identification of important and tangible scientific questions and ideas, formulation of mechanistic hypotheses and critical testing of predictions.

In addition, microbial ecologists are traditionally preoccupied by techniques and, like other disciplines, has been heavily influence by the availability of molecular and 'omics' techniques. History teaches us that the wonderful techniques of today and the descriptions they provide will soon be outdated and replaced. This is exemplified by the relentless progression from 16S rRNA gene analyses, through transcriptomics, proteomics, metabolomics, metagenome-assembled genomes and network analysis. Each has been proposed as the solution to increasing understanding in microbial ecology, while explaining the glaringly obvious deficiencies of their immediate antecedent. Often, their use has persisted even after attempts at validation have demonstrated their inability to fulfil their supposed potential. Witness use of functional gene abundance as a measure of activity and use of network analysis to identify microbial interactions in complex, spatially structured natural environments.

Again, this does not mean that these, and other new, amazing and seductive techniques, are not of value but their value is determined by their ability to address important questions and test important hypotheses. In many cases, these techniques have been embraced by those

Accepted Article

Accepted Article

addressing scientific questions. However, in most they have been used without serious consideration of how they might investigate specific phenomena and hypotheses, or their role in critically testing hypotheses. The intense focus on techniques has legitimised descriptive technique- and data-based studies, and publications, at the expense of scientific studies. A major concern, therefore, is that this has created a generation of researchers who uncritically accept this approach and who are now supervising researchers, reviewing papers and proposals and deciding on science policy, with little appreciation of scientific questions.

The solution

The problem described above should not be exaggerated. Many researchers do ask interesting questions and do test hypotheses and microbial ecology has advanced significantly in recent years, in part due to remarkable advances in technology. The concern is that the rate of advance is significantly limited by the lack of awareness or consideration of the need to adopt a scientific approach in the majority of studies. Fortunately, the solution is in our own hands. As practising microbial ecologists, we review funding applications and review and edit research papers. We can therefore ensure that scientific principles and approach, outlined in the scope and aims of funding programmes and journals, are not disregarded. In reviewing funding proposals, we should look for identification of unexplained phenomena, why they need to be explained, clear scientific questions, potential explanations and hypotheses and how they will be addressed and tested. Without these, requested resources, staff cannot be justified. It is reasonable to ask reviewers to assess the scientific merit of a research proposal, but impossible, by definition, to rank applicants on the basis of who is the luckiest. Equipment requests never include good luck charms.

As editors and reviewers of submitted papers we also require identification of questions, explanations and the degree to which experimental data have significantly advanced understanding, by either supporting or rejecting explanations and hypotheses. Papers without clear scientific aims should be rejected, as their absence prevents assessment of the success of a study. This does not preclude papers on development of methods, or development of hypotheses, but the former should indicate which scientific questions they might help answer, while the latter should demonstrate how they could be tested empirically.

Importantly, as researchers we should have a clear idea of the phenomenon(a) we aim to understand and the scientific questions we are asking. If asked to describe your research in a single sentence, would you describe your research question, rather than describing the techniques you are using? We therefore need, at all stages of our research career, to ignore the elephants in the room, and be brave enough to look for interesting but maybe difficult questions, propose bold and risky hypotheses, test them critically and be prepared for them to fail. This, rather than relentless aimless accumulation of data, has and will provide real advances in microbial ecology.

References

Chalmers, A.F. (2013) What is this thing called science? 4th edn. St Lucia, Australia: University of Queensland Press.

Craver, C.F. and Darden, L. (2013) In search of mechanisms. Discoveries across the life sciences. Chicago: University of Chicago Press.

The Stanford Encyclopedia of Philosophy. Ed. Edward N. Zalta. World Wide Web URL: https://plato.stanford.edu/. The Metaphysics Research Lab, Philosophy Department: Stanford University.

Prosser, J.I. and Nicol, G.W. (2012) Archaeal and bacterial ammonia oxidisers in soil: the quest for niche specialisation. *Tr Microbiol.* **20:** 523-531.

Popper, K. (1959) The logic of scientific discovery. New York: Basic Books.

Popper K. (1963) Conjectures and refutations. 1st edn. London: Routledge & Kegan Paul.

Accepted Article

Prosser, J.I. (2020) Putting science back into microbial ecology: a question of approach. *Phil Trans Roy Soc B* **375**: 20190240. http://dx.doi.org/10.1098/rstb.2019.0240.

Pasteur, L. (1854) Lecture, University of Lille, 7 December 1854.

Accepted Article