

## **20 years since Dunedin: The past and future of microbial ecology**

Tiedje, J.M.

Center for Microbial Ecology, Michigan State University,  
East Lansing, MI 48824, USA

### **Microbial ecology as a discipline**

Microbial ecology has a long history. The concepts of microbial ecology were at the heart of the pioneering microbiology research of Beijerinck and Winogradsky more than a century ago. But, the assembly of a microbial ecology knowledge base, the coalescence of scientists, especially young scientists, with a new common vision only began in the 1970's. This was led by the first two textbooks on microbial ecology [1, 3], and was followed by the new journal, Microbial Ecology, first published in 1974, the change in the name of Applied Microbiology to Applied and Environmental Microbiology in 1974, and the initiation of the series, Advances in Microbiology, in 1977 [2]. This period initiated the recognition of microbial ecology as more than an *ad hoc* interest but as a distinct discipline. In the 1990's, and especially with the Halifax ISME meeting, we have reached the status of a well recognized, established discipline. Microbial ecology should no longer be considered as an interest group under the umbrella of ecology or of microbiology. To exist under these historical disciplines limits the vision and potential of a field. Our field should devise and implement its own unique program.

### **What perspective have we gained from our past?**

The title of this paper recognizes the formative event for microbial ecology, the first International Symposium in Microbial Ecology (ISME) which was held in 1977 at the University of Otago in Dunedin, New Zealand. A look at the history begs the opportunity to analyze at the current era and to speculate about the future, which I will do. But first, the history. I would particularly like to call attention to the efforts of Professor Margaret W. Loutit of the University of Otago who was the organizer of the first ISME symposium. She and her colleagues in the New Zealand Microbiology Society first recognized that it was time to gather together people with a common interest in microbial ecology and that doing so would help build a common knowledge base, stimulate new synergies, and result in common research themes. Over 400 scientists from 30 countries attended the Dunedin meeting. Three in this Halifax audience, besides myself, attended. Clearly the discipline has grown successfully since Dunedin; this meeting is four times larger and populated by a completely new generation of scientists. Please join me in thanking Margaret Loutit for the vision and energy she showed in founding the ISME meetings and through her other activities to advance the field of microbial ecology. Professor Loutit also served as Chair of the International Committee on Microbial Ecology (ICOME) which has been the formal oversight body that guides these meetings. ICOME is also the founding body for the new International Society for Microbial Ecology established at this Halifax meeting.

#### ***Microbial Biosystems: New Frontiers***

*Proceedings of the 8<sup>th</sup> International Symposium on Microbial Ecology*

*Bell CR, Brylinsky M, Johnson-Green P (eds)*

*Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.*

We are now at the 8<sup>th</sup> ISME meeting. ISME meetings have occurred every three years since 1977 and have rotated among different regions of the world with the goal of stimulating interest of young scientists in those regions in the discipline. The history of the ISME venues follows:

- 0 - Uppsala, Sweden, 1972
- 1 –Dunedin, New Zealand, 1977
- 2 –Warwick, United Kingdom, 1980
- 3 –East Lansing, Michigan, USA, 1983
- 4 –Ljubljana, Yugoslavia, 1986
- 5 –Kyoto, Japan, 1989
- 6 –Barcelona, Spain, 1992
- 7 –Santos, Brazil, 1995
- 8 –Halifax, Canada, 1998
- 9 –Amsterdam, The Netherlands, 2001

I consider the founding of international microbial ecology meetings to not be complete without mention of the efforts of Professor Thomas Rosswall of the University of Uppsala, Sweden, who organized what has become known as the Zero meeting in microbial ecology, because it came before the first formal ISME meeting. This meeting was held at the University of Uppsala in 1972 and was titled “Modern Methods in Microbial Ecology”. The book from that meeting [7] is still in use. Dr. Rosswall also later served as ICOME chair. It is noteworthy that in 1972 as well as in 1998, that methods, especially new methods, seem to remain central to advances in microbial ecology. The importance of methods to our field makes us in some sense more akin to astronomers than to ecologists. We should recognize that new methods are and will for some time be at the heart of the good science needed to advance understanding of the small and diverse organisms that comprise the subject we study.

What was presented in Dunedin? How have we evolved? Two hundred, forty papers were presented at the first meeting (no posters, they were invented at a later time), and 88 papers were published in the proceedings [5]. Of those 88 papers, its most interesting to see what was missing, or in other words, not yet developed. Only one paper mentions rRNA and that was by Professor DeLey, who was pioneering the use of rRNA hybridization to determine species relationships. Only one paper dealt with biodegradation of pollutants and only one paper studied modeling and kinetics. In Dunedin, the most popular new method was the use of fluorescently labeled antibodies. This method had given a tremendous boost to the field since *in situ* autecology was possible for the first time. The papers that were published in the first proceedings fall into the following classes: 26 plant-microbe interactions, 20 animal-microbe interactions, 15 C/N/P/S cycling, 15 public health-related microbial ecology, and only 7 papers on aspects of pollution. Microbial interactions were at the heart of microbial ecology in that era and especially with economically important hosts, i.e., plants, animals and humans. Pollution problems were not yet a significant source of research funding. The growth of the public's interest in environmental issues, and with it research funding, occurred in the 20 years since Dunedin. Topics such as hazardous waste remediation, groundwater quality, waste water clean-up, global warming, land use effects, and the environmental impact of

pollutants has all occurred since Dunedin. The new research funds that derived has had a major impact on the direction, scope and size of the microbial ecology community.

I would characterize the first decade of microbial ecology as focused on organism interactions and I'd characterize the second, the 1980s, as focused on kinetics and modeling. This period coincided with and was probably dependent on the availability of the personal computer. The ISME meetings in 1983 and 1986 had a strong representation of mathematical microbial ecology. The third decade, that of the 1990s, has been dominated with the infusion of molecular methods. Unfortunately this appears to have come at the expense of the mathematical skills the field had advanced in the 1980s. I mention this because I think we will need those skills in the near future to assemble the information from the molecular studies in a synthetic, functional and predictive manner. Our current students, now often focused on molecular methods, should not neglect their basic mathematical training and their willingness to begin to assemble their results in a formal quantitative context.

### **What now? The age of communities.**

I characterize the 1990s as the age of communities in microbial ecology. We now have the methodology to make this a tractable subject of study. At the 1992 ISME meeting in Barcelona, Professor Kevin Marshall in a plenary address, identified the study and understanding of microbial communities as the microbial ecology theme of the future [6]. He was insightful. We are now in the middle of the community era. We now can assess community composition and structure, and at this meeting for the first time, we are beyond the survey. We are: (i) identifying meaningful patterns in community structure and their causes, (ii) have several well characterized systems that are positioned for in depth cause and effect, mechanism oriented studies, and (iii) we are beginning to link function to community composition. To complete the community era, we need to finish linking function to community structure so that we can understand the biological underpinnings of function and hence predict function, and we need to learn how to manage communities. If we can effectively do the latter, at least for a few types of communities, the practical payoff can be very high.

Community structure is a product of two components: the **inoculum**, i.e. the initial populations present in the habitat which is the result of natural or human induced dispersal, and **selection**, i.e., the conditions that provide for colonization and growth of the successful competitors. Most communities are a result of these two forces. For the microbial world, however, we have a problem. Some members of the community may be transients and not truly adapted to grow and reproduce in that habitat because some microbes can survive for very long periods of time outside sites where they can successfully compete.

Any era in a field should be driven by major questions to be answered. I identify some below that I consider central to the current community era:

1. Which environmental parameters are most important in selection? Can a predictive relationship be discerned between environmental parameter and population?
2. How important are biotic interactions vs. abiotic factors in determining the resulting community?
3. Can differences in community function be assigned to differences in community structure?

4. What determines the richness of communities?
5. What is the extent and the degree of novelty of non-culturable members?...their functional roles?
6. How can transients be distinguished from community inhabitants?

Answering some of these questions will go a long way in making this a successful, productive era in microbial ecology.

As I mentioned above, methods have had a central role in microbial ecology. Because of the measurement challenges we face, I think it is not wise to become too attached to one method or approach. Most methods have short comings, hence complimentary features of two or more methods can offer powerful advantages. In Table 1 I identify classes of methods and a few examples of each. Most important, however, is the consideration of the different method classes since each measures very different organism features and each has its advantages and disadvantages. Phenotype is important because this class most closely relates to function. BIOLOG™ is mainly designed to distinguish among and identify bacteria, rather than to measure major ecologically important traits. Nonetheless, phenotype based methods are ultimately the most important of the suite in adding functional insight.

**Table 1.** Some of the tools for microbial community analysis and their relative advantages

| Type of method  | Entire communities | Reliability | Functional interpretation | Speed | Ease (skill level) |
|---|--------------------|-------------|---------------------------|-------|--------------------|
| <b>Phenotypic</b><br>BIOLOG                                 | +                  | +           | +                         | +++   | ++++               |
| <b>Chemotaxonomic</b><br>FAME, PLFA                         | ++                 | ++          | ++                        | ++    | +++                |
| <b>Genetic</b><br>16S ARDRA<br>Sequencing, probes<br>T-RFLP | +++                | +++         | +++                       | +     | +                  |
| <b>Morphology</b><br>Image Analysis                         | ++                 | ++          | +                         | ++++  | ++++               |

‘+’ = Limitation

‘++++’ = Most advantageous

Chemotaxonomic methods are especially important where molecular markers provide sensitive detection of microbial groups, and in some cases reveal activity information. They are also often quantitative. Such markers exist for some microbial groups, but many more are needed for this approach to be comprehensive for natural communities. Not all classes of markers are yet explored and I’d expect the further advancement in this area to follow advances in analytical chemistry.

Genetic methods have been a contemporary favorite. Without doubt these are powerful and important additions to our field. My concern is that we can be too infatuated, too blinded with methods of the moment. rRNA based methods could be in danger of becoming such an example. At this meeting, I’d estimate that perhaps 4,000 new SSU rDNA sequences are behind the presented work. We need to think beyond the sequence and beyond the correlations of sequence data to mechanistic understanding of populations

and their dynamics. Importantly rRNA, being a conserved molecule, is not reliable for differentiation of the species and subspecies level. This is the level where many of the traits important to ecological success reside. Hence, correlation of ecotype and function to rDNA classes is weak. I'd encourage some attention to the next step, differentiation of populations at the species and ecotype level.

Morphology, including the power that comes with dye chemistry and associated probes makes microscopy methods powerful and warranting further development. Microscopy coupled to advanced image analysis is potentially the fastest way to assess complex communities. At our Center for Microbial Ecology, the team working with Dr. Frank Dazzo, a microbiologist and Dr. Anil Jain, a computer scientist, have recently perfected an advanced image analysis system that recognizes the 11 basic microbial morphotypes, the critical step in advancing image analysis for microbiology. This tool coupled with dye chemistry should provide fast, efficient and quantitative characterization of microbial communities.

Table 1 also provides a general indication of the advantages and limitations of the different methods. The score shown is from my personal impression and is likely debatable. The point I want to make, however, is that a variety of characteristics need to be considered in evaluating the choice of methods for community analysis. Published papers usually focus only on the scientific results and not other practical matters like cost, skill level, number of replications possible and reliability.

Most of us now recognize that microbial communities generally exhibit a high level of diversity, much higher than previously assumed by what was revealed through classical microscopy and basic culturing techniques. Even supposedly simple communities such as limited substrate bioreactors and drinking water are more microbially diverse than previously thought. The most complex community appears to be soil. DNA-DNA reannealing studies, screening of rDNA clone libraries and more vigorous cultivation attempts have all shown microbial diversity to be very high. One thousand to 10,000 species per gram is not an unreasonable estimate. This compares to only 4,200 validly described prokaryotic species, most of which are not soil organisms. This comparison shows that we have far to go for any comprehensive characterization of the soil microbial community. Why is soil microbial diversity so high? Several factors may contribute including the high diversity of food resources; a rather stable, protective, even ancient, environment; and what appears to be a high degree of spatial isolation that reduces competition, thereby maintaining less competitive members. Recent work by colleagues, Dr. Tsutomu Hattori and Klaus Nüsslein using environmental scanning electron microscopy has identified very stable soil microaggregates of 100 to 600  $\mu\text{m}$  that harbor microbial colonies interior to the aggregate. This interior community may be protected from outside competition and predation for perhaps hundreds of years. A rather eloquent impression of the soil habitat was provided by a graduate student, Thomas Hill of the University of East London. I think his description was so appropriate I quote it here:

“On a human-eyed scale, the soil for a bacterium must be like living in a 30 km-high, crumbling, dark Bladerunner-esque city that is often deluged with water, packed with garbage, and full of all manner of modest and badly ventilated dwellings. Apart from the extra dimension, pretty much like London in winter in fact. The only difference with our fine city is that I

suppose the landscape would be peppered with catabolic fires around root tips, within and in the wake of worms, and following the death of roots and soil organisms, creating spectacular opportunities for several trophic groups. So, maybe spatial and temporal separation coupled with much randomness and the non-linearity of many key soil functions create/permit such equitability.” [4]

### **What will we see at ISME-15 in 2019?**

If we try to look ahead 20 years, what might we expect to see? Of course this is highly speculative and dependent on advances in other fields. In 1977 we likely could not have predicted the impact of the personal computer or the molecular methods, but perhaps we could have predicted the impact of environmental concerns which has shaped the topics we now address. Given this caveat, I'll try to predict some themes of ISME-15.

#### **What will we see in 2019?**

- **Microarrays to assess community composition**
- **Microarrays to assess community expression**
- **A three dimensional physical and functional model of the (e.g. soil) niche**
- **An interface with computational biology**
- **An rRNA bush, not a tree**
- **Still no prokaryotic species definition**
- **No new SSU rRNA sequences**

I suggest that the microarray technology and genome sequencing will have a major impact on our field. We deal with complex diversity and we are basically reductionistic and mechanistic in our orientation. The array technology or what will succeed it are tools for complexity and reductionism. I'd expect we will have microarrays that assess community composition and others that assess which genes are being expressed, probably within the next decade. Perhaps there will be a way to even link these measurements to real-time control of conditions that influence communities, such as in the management of bioreactors.

The next level of integration of composition and expression information is with the communities' physical and chemical environment. With computational methods it might be possible to describe a 3-dimensional physical and functional model of a microbial niche; the soil aggregate would be the ultimate challenge. This goal of synthesis of complex information brings us closer to the computational sciences. This begins with understanding competition, the microbial model for this ecological question has many advantages over the macroecological models in that our system is simpler, fewer genes and traits, and potentially explorable at the genetic level by mutation.

rDNA sequence analysis will have approached its ultimate end in 20 years if not before. We will likely see the phylogenetic tree as a bush showing a continuum of many types of species. A prokaryotic species definition is likely still to be controversial and its resolution perhaps avoided for sociological reasons rather than scientific. I list that there will be no new SSU rRNA sequences to be found. This is partly in jest. More likely there will still be a few sequences to be found, but the interest in this methodology may have declined long before 2019, perhaps supplanted by genome sequence information.

In summary I'd like to conclude by emphasizing that the state of our discipline has never been stronger, our future is bright because many major challenges of society over the next decades have their root in microbial ecology. We have grown substantially over the past 20 years, become an independent discipline and have a core knowledge base. Importantly, we should advance questions important to microbial ecology as a distinct field, not following the trends of ecology or microbiology. The shift at this meeting from our past, loosely-organized structure of ISME meetings under ICOME to a formal society, the International Society for Microbial Ecology, is an important step for future growth of our field. It gives us more influence in shaping our goals, obtaining funds and in communicating among ourselves and with the outside world. Hopefully ISME-15 will show an even further level of growth and evolution of microbial ecology.

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