

A RESEARCH AGENDA FOR MICROBIAL OBSERVATORIES: A 1999 PERSPECTIVE FROM INTENSIVE LTER PROJECTS

The LTER Committee on Microbial Ecology¹

INTRODUCTION

Microbes are essential for the functioning of ecological systems. Vital microbial processes include decomposition and mineralization, many aspects of the nitrogen cycle (e.g., nitrogen fixation, nitrification, denitrification), plant nutrient uptake via mycorrhizal associations, microbial nutrient immobilization, the production of biomass to fuel ecosystem food webs, and the breakdown of toxic materials. While ecologists have developed methods to measure many of the rates of these processes, most have effectively ignored the microbes themselves. These they treat as a "black box," an approach that ignores possible controls of processes by species or community interactions. In other words, ecologists do not deal directly with many aspects of the ecology of important microbes.

It is now time to move to a new level of knowledge of ecological systems. This new level of understanding of these ecological systems can only be achieved through a better understanding of microbial abundance, distribution, dynamics, communities, and of how these communities function and are controlled.

Some of the improved understanding can be achieved by the imaginative use of existing methodology applied uniformly across a wide variety of ecosystems. Some will only be achieved by the development of new methods that will arise from the rapid advance of molecular and physiological microbiology. It is now clear that the application of new molecular techniques to ecological questions must include development rather than simply direct transfer. Microbes in their natural habitats are often inactive or active at so low a level that existing methods, developed for in vitro populations, often do not work.

MICROBES IN ECOLOGICAL SYSTEMS

The goal of microbial observatories should be to understand microbial functioning and controls in ecological systems. Within this goal the committee recommends topics under the following themes:

- Measurement of microbial production and biomass. Additional techniques are needed that do not suffer from the limitations of current methods, and these and existing techniques need to be standardized for use across the whole range of ecological systems. In what ways are microbial productivity and primary productivity linked? What are the relationships between microbial biomass and the rates of microbial processes? What proportion of microbial energy is derived from fresh photosynthate from algal cells or plant roots vs. dissolved organic carbon from positions higher in the hydrologic flow path vs. structural material stored in soils and sediments?

- **Microbial community composition.** Unlocking the black box requires better ways to describe and enumerate taxonomic units of operational value. rRNA-based tools have become a powerful means to describe microbial communities. There are many different approaches to the use of this basic tool but all of them are limited by the lack of a comprehensive probe and primer suite for uncultured members of environmental communities. Plus, existing techniques are slow and cumbersome on the scale needed to address meaningful ecological questions. Can species probe and primer sequences be advanced to answer field-based questions? For example, how are microbial species distributed and what controls their abundance? Are there geographic limits to distribution or are all species always present? Are the microbial species that have been well-studied in the laboratory actually present, active, and important in nature or have we been studying laboratory weeds? What is the best way to characterize microbial communities without identifying every species? Is there seasonal, annual, decadal succession of microbial communities as environments change? What is the role of microbes in facilitating recovery of ecological systems from disturbance?
- **Assessment of functional gene diversity through molecular tools.** Functional genes are less conserved than rRNA genes, which necessarily leads to a need for different molecular tools. There are now enough sequences of important functional genes that we can begin to evaluate functional diversity. Examples of such gene families are ammonium monooxygenase (nitrification), methane monooxygenase (methane oxidation), dissimilatory nitrite reductase (denitrification), nitrogenase components (nitrogen fixation), Rubisco (primary production). Can profiles of these gene families be a means of comparing sites over time and space? Could current molecular techniques, such as DGGE, T-RFLP, and hybridization arrays, be a means to evaluate functional gene diversity?
- **Development of techniques for measuring activity of individual cells.** What percentage of the total population are active at any time? Are there reasonable ways to carry out autoradiography of microbes in situ? Will mRNA probes work for specific enzymes in microbial communities? Can in situ PCR of functional mRNA be developed to be widely useful for natural microbial communities?
- **Linking microbial communities to processes.** Once the black box is unlocked how much does its composition matter to rates of microbial processes? Do changes in microbial communities imply changes in microbial processes such as respiration and denitrification, or is there a great deal of functional redundancy? How are microbial food webs affected by changes in microbial communities and by the activities of animals such as rotifers, protozoans, nematodes, and annelids?
- **Quantitative modeling.** Mathematical modeling has proven to be a useful tool for integrating ecological knowledge in many ecosystems but there has been little success in modeling microbial systems. To what extent does information about microbial community composition need to be incorporated into quantitative process models? What knowledge do we need in order to effectively model microbial communities?

CONCLUSION

Substantial effort has been devoted to understanding the role of microbial biomass, productivity,

and process rates in soils, sediments, and other important habitats in terrestrial and aquatic ecosystems. Too little of this effort has been directed towards long-term, spatially distributed investigations that allow us to draw synthetic conclusions about controls on the abundance, distribution, and importance of microbes to ecosystem function. In part this lack is a result of unavailable technology – e.g., only recently have many molecular techniques become sufficiently refined to be applied to environmental samples. It is now time to apply a balanced approach to important questions about the roles of microbes in ecological systems. Such an approach should include the further development and standardization of existing, conventional techniques for studying microbes and microbial processes as well as the development of emerging molecular and physiologically based approaches, such that they can be used *in situ* or with samples direct from the field. In this way we can hope to gain a fundamental understanding of the role of microbes in a broad range of ecosystems and of their response to disturbance.

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