Microbial Eukaryote Diversity and Biogeography

Recent insights into protistan ecology are fueling a debate over their diversity, distribution, and also the species concept

David A. Caron, Rebecca J. Gast, Peter D. Countway, and Karla B. Heidelberg

Microbial ecology has come of age. Microorganisms of all types are essential participants in virtually all biogeochemical processes on earth, a realization that has developed steadily during the last half-century. Moreover, the diversity of bacteria and archaea, revealed by oceanographers and marine bacteriologists through the cloning and sequencing of genes from natural water samples beginning in the 1990s, is far greater than traditional culture-based methods had previously revealed.

More recently, the early focus on bacterial and archaeal diversity has broadened to include the protists, those single-celled eukaryotes that play fundamental roles in energy flow and elemental cycling in aquatic and terrestrial ecosystems. The photosynthetic protists, or microalgae, are producers of substantial amounts of organic matter, while the heterotrophic protists, or protozoa, are important consumers of bacteria, archaea, and other eukaryotes. Despite a wealth of information on the basic physiology of many protistan species, genetic approaches are revealing a previously unknown and presently uncultured diversity among these species, similar to findings in bacterial and archaeal ecology. Moreover, we know relatively little about how these eukaryotic species are organized into functional assemblages or how changing environmental conditions affect those assemblages. Growing information about their diversity and distribution makes protists the subject of lively arguments over their ecology and how they should be divided into species.

Summary

- Genetic studies of environmental samples are revealing previously unobserved protistan taxa, new lineages, and rich diversity.
- Experts disagree over the total diversity of protistan forms and distributions in nature.
- Central to understanding protistan diversity and its distribution is the species concept.
- New methods for examining protistan species richness are altering our perception of community assembly and how it responds to environmental changes.

New Approaches Reveal a Hidden Community of Protists

Information assembled from the cloning and sequencing of protistan genes, especially small subunit ribosomal RNA (18S) genes, is changing perceptions of eukaryotic phylogeny and continues to generate new proposals regarding the evolutionary relationships among eukaryotic taxa. When this same analytic approach is applied to environmental samples of protistan assemblages, the results extend discoveries in environmental bacteriology from the 1990s.

Phylotypes from environmental clone libraries of protistan assemblages indicate numerous taxa without corresponding morphotypes among cultured, sequenced species. For example, this approach revealed two new lineages discovered within the Alveolata, which is a morphologically diverse group that includes the dinoflagellates, ciliates, and apicomplexans. Several novel lin-

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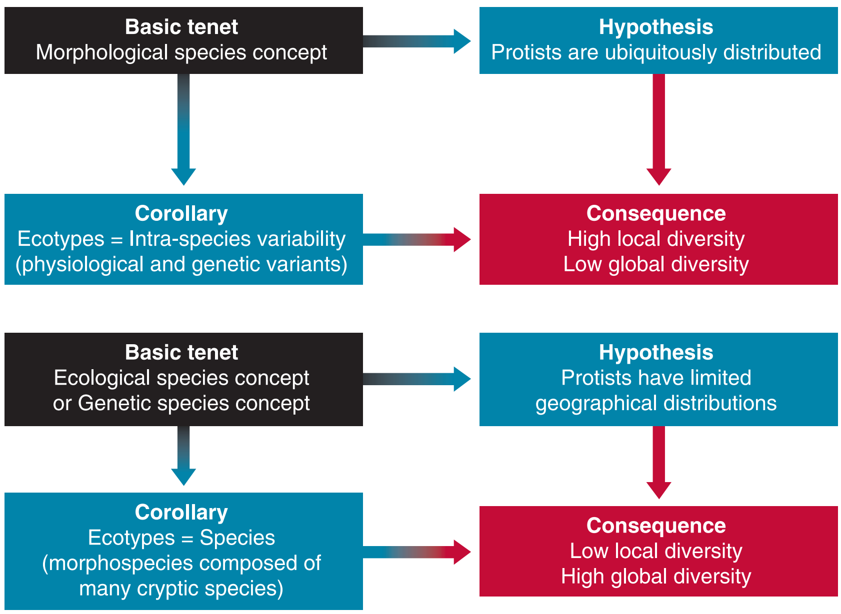
FIGURE 1



Opposing tenets, hypotheses, and consequences regarding the species concept, global diversity and distribution of protists. Acceptance of the morphological species concept and the presence of similar morphological forms worldwide has led many to speculate that protistan species exhibit ubiquitous dispersal, and that total global diversity among protists may be relatively low while local diversity can be quite high. In contrast, the recognition of considerable genetic and physiological diversity within protistan morphospecies has led others to propose the existence of many cryptic species within a morphospecies. Protistan 'species' may therefore exhibit considerable endemism and global diversity may be extremely high.

eages have also been documented within the Stramenopila, which is a group that includes the diatoms and other well-known phototrophic and heterotrophic protists.

Other unusual findings include gene sequences that indicate the presence of monothalamous foraminifera in freshwater ecosystems, overturning the notion that they are restricted to marine ecosystems, and also the presence of kingdom-level eukaryotic microorganisms in anoxic ecosystems. If confirmed, this latter finding would be no less significant than discoveries of the early 1990s that revealed that archaea are widely distributed in the oceans (*ASM News*, October 2003, p. 503).

Finding DNA sequences in environmental clone libraries that do not match those from cultured taxa should not be so surprising. Only a small fraction of protists is represented in culture collections, and the discrepancy is particularly glaring for protozoa. However, the sheer numbers of uncultured protists indicated by novel phylotypes in clone libraries is staggering, and the phylogenetic distances between these sequences and those for known species, classes, phyla, and perhaps kingdoms is remarkable. Methodological artifacts notwithstanding, these findings provide evidence that we have cultured only a fraction of the protistan species present in nature.

What do these newly discovered protistan species look like, and what are their ecological roles? Researchers are making some progress as they try to address these questions. For instance, some recently identified novel stramenopiles are composed largely of small, nondescript, heterotrophic flagellates that do not compete well with other protists in nutrient-enriched media, perhaps accounting for why these taxa were so long overlooked.

In the case of two novel alveolate lineages, one contains several previously described parasitic dinoflagellates. Perhaps these lineages contain largely parasitic forms, which could explain why these novel protistan groups went unnoticed for so long. Further, the novel alveolates that associate with deep-sea benthic communities might be parasitic taxa that cause mass mortalities among animals inhabiting hydrothermal vents. However, that possibility remains conjectural.

Environmental bacteriologists now recognize that only a fraction of the bacteria in nature are culturable using established approaches. Protistologists are now struggling with the same realization. Indeed, some investigators still doubt recent claims that many of the novel rDNA phylotypes being observed through cloning and sequencing represent previously undescribed, uncultured protistan taxa. However, the cumulative evidence is changing this mindset, while raising uncertainties regarding our understanding of microbial eukaryote diversity.

Some Experts Say Protistan Diversity Is Limited

Large numbers of new genetic signatures, albeit lacking names or linked morphologies, have in-

Caron Pursues Protists while Splashing through Ponds or from Deep-Sea Submersibles

Oceanographer David Caron spent parts of his youth splashing through local ponds and exploring the woods in the eastern part of Massachusetts, about an hour south of Boston. "I think anything and everything alive interested me," he says. "Like so many other kids, I grew up mesmerized watching Jacques Cousteau on TV. I guess that did it for me, I was going to be an oceanographer." As a youngster, he also set his sights on the nearby Woods Hole Oceanographic Institution, where he later spent 14 years as a scientist.

Today Caron, 54, is a professor in the department of biological sciences at the University of Southern California (USC) in Los Angeles. He and his collaborators study aquatic microbial ecology, with a focus on protists, eukaryotic microorganisms. One goal is to provide an accurate description of the roles that protists populations play in producing and using energy, and also in cycling elements in oceanic and freshwater environments.

Many of Caron's experiments are conducted from ships or at land-based field stations. During Thanksgiving week last year, he collected samples while aboard the deep-sea submersible *Alvin* as it traveled along the East Pacific Rise and in Guaymas Basin in the Gulf of California. The primary goal of that brief expedition was to survey protistan diversity "at two contrasting hydrothermal vent areas," he says.

Caron grew up in North Attleboro, Mass., and received his B.S. in microbiology in 1975 and his M.S. in oceanography in 1977, both from the University of Rhode Island (URI). He earned his Ph.D. in 1984 from the joint program in biological oceanography at Woods Hole and the Massachusetts Institute of Technology. Two professors at Rhode Island, now deceased, greatly influenced his career path, prompting his realization "that science was a career of which I would never tire," he says. The first, R.D. Wood, an aquatic botanist, "taught me the joys of sloshing through bogs and ponds—which wasn't a stretch for me—in search of herbarium specimens-to-be."

However, Caron claims that John McNeil Sieburth, then a marine microbiologist at the Narragansett Bay campus of URI, was responsible for launching his career. "I had the good fortune to do a couple of semesters of directed study with John in the last year of my undergraduate degree," he recalls. "Many who knew John would consider my luck 'dubious,' but I got along fine with John, [who] was eccentric—an understatement—but never ever boring," he adds. "I got hooked working with microalgae and protozoa in his lab. I even named a dog after him. Sieburth, the yellow Labrador—also now deceased—who was as much of a free spirit as his namesake."

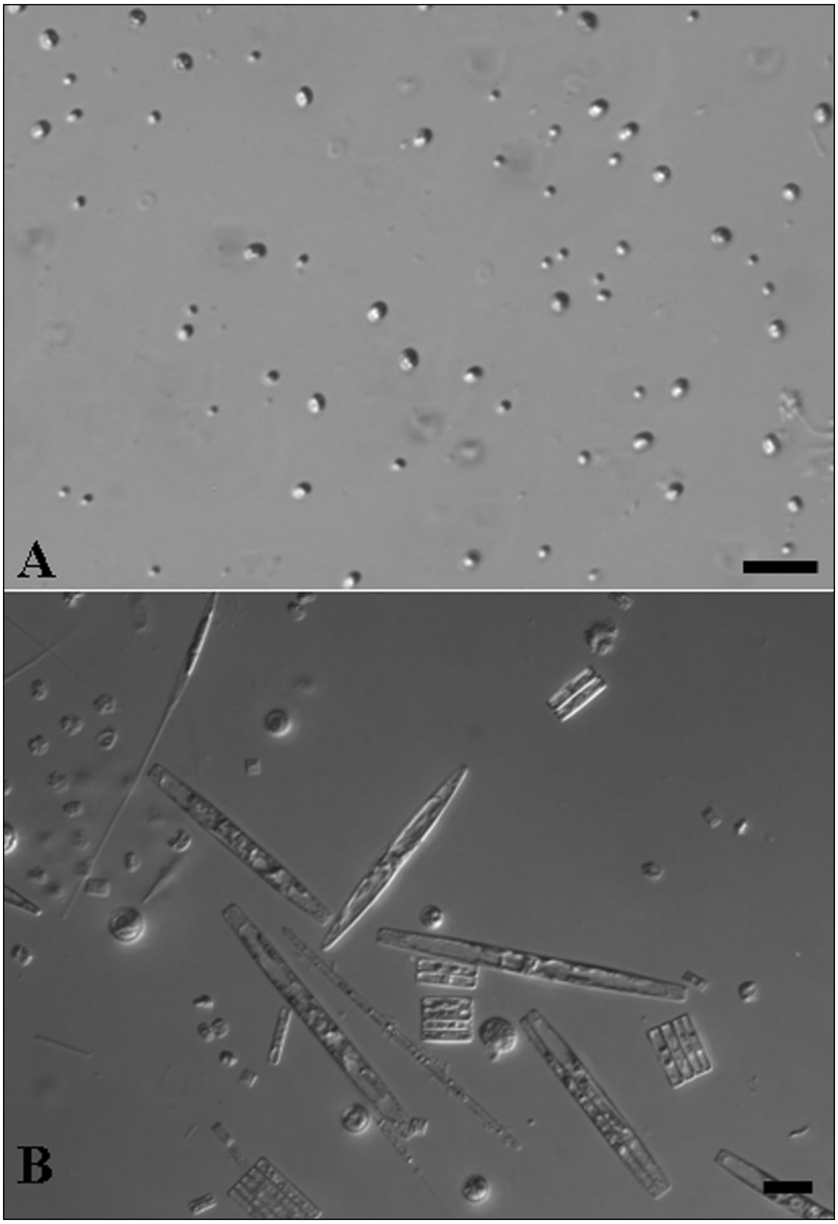
After finishing his doctorate, Caron served as associate research scientist at Lamont-Doherty Geological Observatory at Columbia University from 1984 to 1985. In 1985 he became an assistant scientist at Woods Hole; in 1989, an associate scientist, and, in 1999, a senior scientist. He joined USC in 1999, where he later served as chair of biological sciences from 2003 until 2006.

Caron's wife Sigi runs a consulting company for medical device producers. Their daughter Zoe, a seventh grader, "seems to be growing up with the same genes that I inherited that give rise to love and admiration of nature, whatever genes those might be," he says. "She is a true-blue nature lover, no question. It will be people like her who save our planet."

Caron spends much of his spare time outdoors – in the mountains, in the desert, on a lake, on the ocean. "Anywhere outside – the more biology the better," he says. "Over the years, I've spent more hours snorkeling and scuba diving than I can count, but in recent years I've really found hiking the Sierra Nevada mountains a tremendous amount of fun, and pulling out the occasional trout." He also likes working around his house, he adds. "I've done enough home repairs, painting, flooring, woodwork, that I sometimes feel I could easily have been a carpenter—and probably would have enjoyed that career also."

Marlene Cimons
Marlene Cimons is a freelance writer in Bethesda, Md.

FIGURE 2



How many species? The morphospecies concept that dominates protistan taxonomy is not perfect. Upper panel: A mixture of five cultures of minute (<5 μm) marine algae. Morphology alone is virtually ineffective at differentiating these physiologically distinct taxa that span three classes of algae. Lower panel: Morphologically distinct taxa of phytoplankton (at least 10 species) from the Ross Sea, Antarctica, may harbor even more cryptic species within each morphotype. There is little agreement on the amount of physiological distinctiveness that is needed to warrant the establishment of discrete species. An analogous dilemma exists among bacterial taxomists. Marker bars = 10 μm.

vestigators wondering about the total number of protistan species and their distributions. Efforts to characterize these protists are part of a campaign to evaluate global biodiversity, which ecologists regard with heightened urgency in the face of human population expansion and global climate change.

Protistan diversity and distribution patterns are inextricably linked to new questions about the species concept as it is applied to protists. Until recently, the morphological species concept dominated protistan taxonomy, beginning at least with discovery of Van Leewenhoek's "animalcules" in the 1600s. Subsequently, the ecological species concept, the biological species concept, and even more recently the molecular species concept are being integrated into many descriptions of individual species. Here again, analogies to bacteriology are strong because the species concept for bacteria is proving no easier to resolve (see *Microbe*, June 2006, p. 269–278).

Thus, experts continue to debate what constitutes a protistan species, leading to vastly different estimates of species richness and global distributions of those species. Proponents of the morphology-based species concept contend that protistan species have a low probability of being eliminated from any particular ecosystem because of their small sizes and enormous population numbers. One tends to see the same morphological forms everywhere because species can be widely dispersed and available niches are finite.

For example, Bland Finlay of the Centre for Ecology and Hydrology in Dorset, United Kingdom, and Tom Fenchel of the Marine Biological Laboratory at the University of Copenhagen in Denmark support the notion that protistan taxa are ubiquitously dispersed. To support that view, they cite numerous examples of the same protistan morphotypes being found in diverse and widespread environments, and also note that latitudinal gradients in species richness for larger organisms do not appear to hold for protistan species. Theirs is an appealing hypothesis for many microbiologists because it adheres to the commonly held credo that, for microbes, "everything is everywhere."

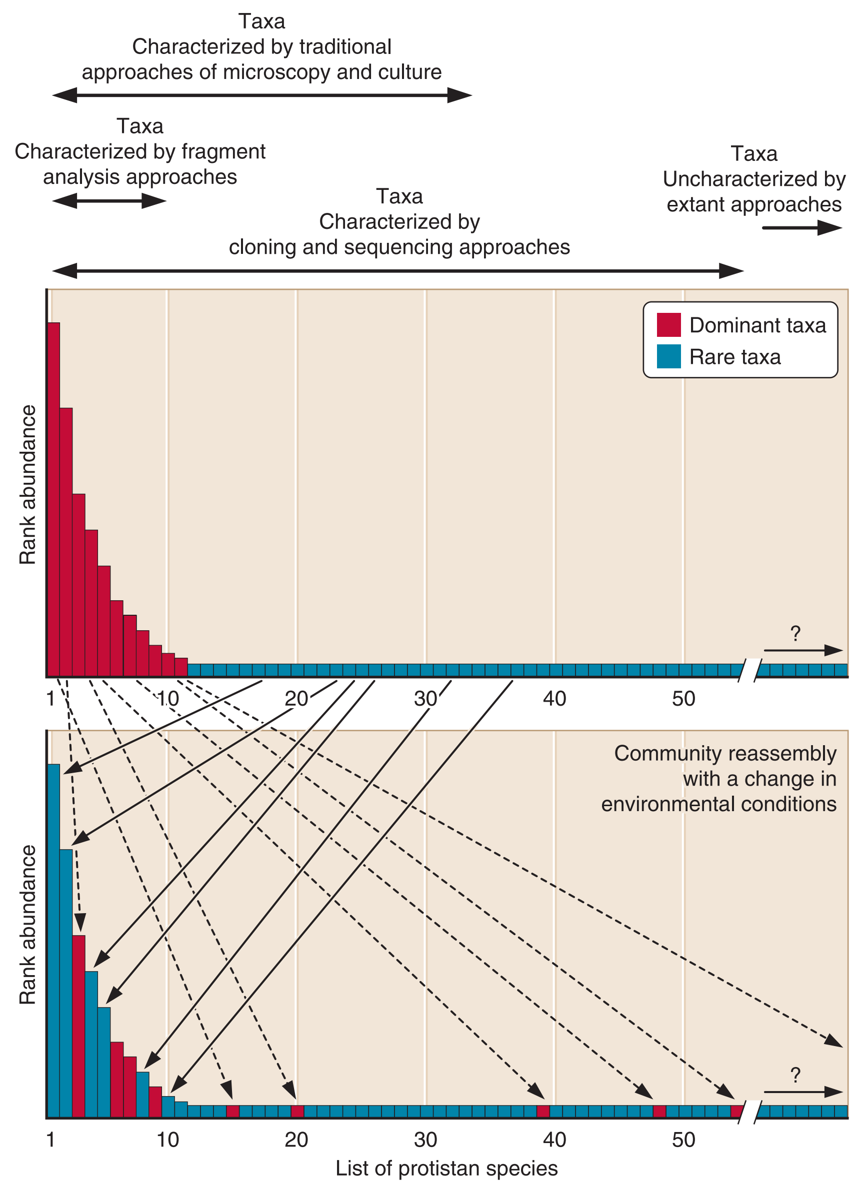
When applied to protistan taxa, this cosmopolitan hypothesis implies that global species diversity among protists is low relative to that of larger organisms, which tend to display a high degree of endemism. There are approximately 20,000 total protistan species, according to Finlay, Fenchel, and their collaborators. Locally, however, species richness for protistan assemblages can be very high relative to that of larger organisms because microbes have a high probability of occupying so many different locations (Fig. 1).

Other Experts Say Protistan Diversity Is Very High

Other experts strongly disagree with those estimates, while citing more recent evidence of genetic, physiological, and behavioral variability within protistan morphospecies and thus raising concerns about relying mainly on morphology when defining species. Some eukaryotic algae provide an example. The minute chlorophytes from lakes in North Dakota carry a wealth of genetic diversity, according to Marvin Fawley of the University of North Dakota. Even cultured strains contain physiologically distinct entities among otherwise morphologically indistinguishable algae, he reports. Similarly, class-level physiological and genetic distinctions are found among minute (<3 μm) eukaryotic algae from marine ecosystems (Fig. 2), and among slightly larger (<10 μm) heterotrophic flagellates from marine and fresh water habitats.

Members of the scientific community continue to debate whether these genetic and physiological findings indicate species-level or intraspecies variability. Faced with a comparable situation, bacterial ecologists coined the term "ecotypes" to refer to physiological variability among strains of bacteria. In similar fashion, Frederick Cohan of Wesleyan University in Connecticut proposed that microbiologists embrace the concept of ecotypes and recognize their importance by designating them

FIGURE 3



Protistan community assembly and reassembly, and the inadequacy of extant methodology to describe the species richness of natural communities. Recent evidence indicates that protistan assemblages can restructure rapidly in nature. Taxa that are rare or undetectable at a given sampling time/location using extant methods of characterization can become dominant members of the community within a few days due to subtle changes in environmental forcing factors. Extant methods can capture the changes in dominant taxa, but it is presently extremely difficult to document the fate of most 'rare' taxa. Full understanding of community structuring and restructuring during environmental change, and its significance for ecosystem function, cannot be attained without the ability to more thoroughly characterize total protistan diversity.

with a Latin trinomial. Some protistan researchers are following a similar path, albeit one that uses different terminology. For instance, intraspecies physiological variability within morphologically defined protistan species prompted some investigators to propose the term "cryptic species," which is analogous to "ecotype" for bacteria. Many experts in this field continue to reject the term and the concept underlying it. However, Wilhelm Foissner of the Universität Salzburg in Austria, who accepts it, estimates as many as 30,000 species of ciliated protozoa alone. His figure for this one subset of protozoa sharply contrasts to the estimated 20,000 *total* protistan taxa from Finlay and Fenchel.

Although a DNA sequence-based taxonomy for protists may help resolve this debate, this approach itself so far is gaining only equivocal support. Reluctance stems in part from our present inability to reconcile DNA sequence information with earlier morphological, biological, and ecological species concepts.

Curiously, for those protistan species that affect human health, investigators generally accept molecular markers as a means of distinguishing among pathogens and parasites. Moreover, protistan ecologists are using genetic approaches because they accelerate the rate at which species can be identified and quantified. There is great hope that taxonomic and other information will soon be melded to yield a molecular taxonomy that is both robust and useful to ecologists.

Now, however, the status of protistan species is becoming further muddled because more and more novel sequences are being uncovered via high-throughput sequencing approaches that are being applied to natural samples. If global protistan diversity is low and sequence variability represents inconsequential differences, then we might attain a reasonably thorough characterization of protistan diversity. However, if the opposing camp is correct, we may be faced with describing a truly unfathomable number of taxa.

Perhaps each side in this debate is partly correct. For instance, maybe some protists have only restricted distributions. Otherwise, how could we rely on taxa such as planktonic foraminifera, radiolaria, and diatoms to characterize water masses and interpret paleoclimatic conditions? Meanwhile, other species may well be globally, or nearly globally, dispersed.

The Species Concept and Assessing Protistan Diversity

The crux of the debate over protistan diversity comes down to what species concept we apply and how much variability we accept as being taxonomically informative. An additional complication is whether we can fully investigate any environment to identify all the species that it contains. While we may not possess that capability, genetic approaches provide the best means for realizing that goal.

Meanwhile, studies to characterize protistan diversity have been largely relegated to documenting the dominant taxa in different environments, applying microscopy and culture approaches as well as DNA sequencing. Instead of resolving the debate, however, such findings seem to be adding to the confusion. One difficulty is that the species richness of protistan assemblages that can be observed using extant molecular biological approaches is on the same order of magnitude determined using microscopy and culture. For example, a reasonably thorough microscopical analysis of a water sample typically reveals dozens to hundreds of species of protists. Current genetic methods tend to provide comparable numbers, but the two approaches often identify very different species within a single sample (Fig. 2). Improved estimates of protistan diversity await technological advances that will provide more thorough analyses of natural assemblages.

Whereas microscopy is at a standstill, genetic approaches soon will expand our ability to appraise microbial diversity. Indeed, the massive sequence information now appearing may settle some of the disagreements over the protistan species concept and, by extension, the debate over species richness and distribution. During the 1970s and 1980s, electron microscopy helped to resolve key disagreements over the taxonomy and phylogeny of protists. Anticipated insights from genetic studies could dramatically improve our ability to characterize and understand protistan diversity when interpreted in concert with traditional taxonomic characters.

Looking Forward

A major goal of studying protistan diversity in particular, and microbial diversity in general, is to understand how ecosystems function. For instance, diversity promotes stability and resilience of communities, according to theoreticians. It would be instructive to learn whether empirical studies support this view. In general, microbial communities provide good model systems for doing this kind of empirical research.

Although empirical studies of protistan communities are in their infancy, some generalizations are beginning to emerge regarding how they are structured and how they adjust to environmental change. According to one theoretical framework for microbial eukaryotic diversity, rare species within a habitat may become common in a different habitat or following an environmental change, with the overall expectation that community processes continue relatively uninterrupted (Fig. 3).

This simple, intuitive hypothesis of rapid community restructuring has appeal because it predicts a great deal of functional resilience of microbial communities in the face of environmental perturbations. However, there are relatively few empirical tests of this premise at present. If true, it leads to the question of how much environmental change is necessary to overwhelm the physiological diversity within an ecosystem and dramatically alter its function. Given the environmental and climatic issues affecting our planet, the answer to that question may be required sooner than we might like to believe.

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