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Letter to the Editor

Tilting at Windmills: a Response to a Recent Critique of Terminal Restriction Fragment Length Polymorphism Data

Blackwood et al. (1) assert that terminal restriction fragment length polymorphism (T-RFLP) data cannot be used to estimate the species level diversity of bacterial communities. While I do not argue with their analytical techniques, nor the interpretation of their results, their conclusion is essentially moot as T-RFLP techniques are rarely used (nor should they be used) to study the diversity of complex microbial communities at the species level. A more useful test would have been to determine if T-RFLP accurately captures bacterial diversity at coarser levels of taxonomic resolution, for which the technique is undoubtedly better suited. On the basis of their analyses, Blackwood et al. (1) argue that our work (2) should be reinterpreted. However, nowhere in our paper do we indicate that T-RFLP captures diversity at the species level, and for this reason, their analyses have no bearing on our study.

My goal is not to unilaterally defend T-RFLP or related fingerprinting methods; to do so would be foolish given that such methods are rapidly being replaced by techniques that will allow us to compare a large number of communities at a high level of taxonomic resolution. Rather, my goal is to point out that we do science with the techniques we have, not the techniques we might wish to have [to paraphrase the former Secretary of Defense of the United States (". . .you go to war with the Army you have. They're not the Army you might want or wish to have at a later time." Donald H. Rumsfeld, 8 December 2004, Kuwait)]. We could spend our careers identifying every individual species in a handful of samples (currently a Sisyphean task), or we could look for patterns in microbial community composition by analyzing a relatively large number of samples at coarser scales of taxonomic resolution and moving to finer scales of resolution as the appropriate techniques become available. Although T-RFLP may not allow us to estimate diversity at very fine levels of phylogenetic resolution, such was not our goal and there is no reason to assume that the species level is the most appropriate level of taxonomic resolution for comparing levels of microbial diversity.

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Noah Fierer
Cooperative Institute for Research in Environmental Sciences
University of Colorado
Boulder, CO 80309-0216

Phone: (303) 492-5615
Fax: (303) 492-1149
E-mail: noah.fierer@colorado.edu

Authors' Reply

We previously showed that diversity indices applied to T-RFLP data tell us little about the diversity of the underlying community (2). Fierer has correctly drawn attention to the fact that our analysis was focused on a phylogenetic level of resolution that is often construed as being approximately equivalent to "species" (97% 16S ribosomal sequence similarity). We feel that this is appropriate because it is the phylogenetic level most often associated with diversity indices and the term "diversity" in ecology and is the level of concern in most related ecological theory.

Fierer suggests that diversity of T-RFLP profiles may correspond with true diversity of communities at a coarser level of phylogenetic resolution. We are not aware of data or arguments to support this position, but it would be interesting if this was tested by simulations or empirical data. In the meantime, given the fact that several studies point to problems with this approach (1, 2, 3, 5, 6), it seems unwarranted to put faith in this particular interpretation of T-RFLP data. It is almost reminiscent of the wishful interpretation of data that led the former U.S. Secretary of Defense to the position where he was forced to make the statement that Fierer has paraphrased. While it is true that we do science with the techniques we have, it is important that the limitations of these techniques are acknowledged so that results can be interpreted properly. There is no question that T-RFLP is a sensitive method to compare microbial community composition, and the work of Fierer and Jackson (4) is still interesting despite the more limited interpretation of their data.

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Christopher B. Blackwood*
Department of Biological Sciences
Kent State University
Kent, OH 44242

Donald R. Zak
Department of Ecology and Evolutionary Biology and School of Natural Resources and Environment
University of Michigan
Ann Arbor, MI 48109-1048

Jeffrey S. Buyer
Sustainable Agricultural Systems Laboratory
USDA-ARS
Beltsville Agricultural Research Center
Beltsville, MD 20705

*Phone: (330) 672-3895
Fax: (330) 672-3713
E-mail: cblackwo@kent.edu