

Using Microbial Ecology to Teach Experimental Design and Sampling Methods

Mary E. Allen and Ruth A. Gyure

Exercise 2: Instructor Guide Community Structure and Organization

Materials needed (per group of two to four students)

- Printout of sample community diagram A, B, C, or D (Word versions are included in exercise). These are necessary and must be printed in color.
- 1 Sharpie or other permanent marker
- 3 plastic transparencies, same size as community diagram (8.5 x 11")
- Data sheet (printed out or electronically accessed on computer)

Introduction

Characteristics of microbial communities that require special sampling consideration have already been mentioned in the general introduction for this activity. Exercise 1 introduces the concept of diversity and one of the ways to measure it after taking samples from a simulated community. The bag community is of course unrealistic in many ways. An important feature of the bag community is the way the items could be thoroughly mixed between each sample. Of course in nature, microbes and other organisms are arranged, clumped, partitioned, etc., based upon many factors that affect them: availability of sunlight, nutrients, and water; interaction with other organisms; presence of antagonistic compounds or conditions; oxygen level and overall atmospheric pressure; and more. Organization and arrangement of cells will also vary temporally. Temporal changes may be rapid (exponential growth and competition in a rich broth, for example) or slow (seasonal or climate-based change).

In this exercise, students will consider how sampling plans are designed to help understand communities at the level of structure and organization, as well as species composition (diversity).

Time

Total time for this exercise is about 1.5 hours. The exercise can easily be done in less than an hour. Time will be longer or shorter depending upon how much time you choose for in-class discussion and data calculation. The questions and analysis may be assigned for students outside of class, some as preexercise questions for thought and others as postexercise follow-up questions. Another suggestion is to assign the questions as part of an online discussion group or blog (e.g., using Blackboard).

Instructor resources

- Instructor information (this sheet)
- Student handout with instructions for the exercise
- Student data sheet (Excel format)
- Four different community diagrams to print (use color)
- Optional: PowerPoint presentation

This can be used throughout the activity. Slides 1 to 10 can be used as an introduction to the exercise. Slides 11 to 15 can be used during the activity; there are projections of the diagrams that can be used when giving instructions. The final slides are helpful during follow-up discussion.

Preexercise questions for discussion and review

1. What is a diversity index and what does it measure?

This question helps reinforce what was learned in Exercise 1. Students should be asked to bring their notes with them from Exercise 1, or both exercises can be done during the same period in which case review is unnecessary.

2. Write the formula for Simpson's index of diversity and explain each of the variables and how the index is calculated.

It is always helpful to review exactly how the index is calculated. Doing this as a group in class assures that each student will be able to calculate the index properly when the exercise is completed, even if the calculation is part of the take-home assignment.

3. List at least four characteristics of microbes that make the study of microbial diversity especially challenging.

These characteristics are given in the introduction to the overall activity. They include challenges of small size (direct visualization may not be possible), differentiation of prokaryotic taxa at species level, tight adherence to surfaces, interdependent relationships, problems of cultivation, and determination of ecological relevance. You and the students may be able to think of many more!

4. When heading out to sample a microbial community, what factors will be important in the planning of your experimental design (brainstorming activity).

Here, help the students to understand that all researchers are limited by realistic constraints, such as how many samples one can afford. Trade-offs are made between the number of samples required in order to validly make comparisons (depends on standard deviation between replicates) and how much time and resource can be allocated. Other considerations include the size and scope of the project, the nature of the questions being asked, the scale of the study area, the nature and scale of interactions among organisms studied, the nature of the system (swirling water or static rock), the technological measuring capabilities available, the type of data that will be collected or measured in the lab if samples are being taken back, etc. This topic should stimulate rich discussion and the examples in the PowerPoint presentation will help to clarify and continue it at the end of class.

5. What is the size of the average prokaryotic cell? How do microbiologists detect and quantify prokaryotic cells? (List at least four general methods and briefly describe how they are done.)

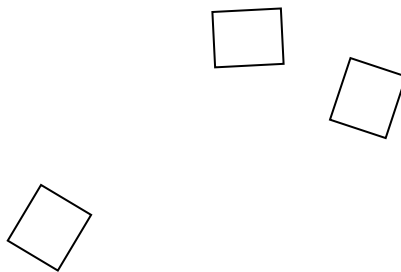
The average prokaryotic cell is between 0.1 and 10 microns, and unicellular eukaryotes are generally 10 to 100 microns—or about 10x larger. Microbiologists use microscopes to view cells directly, and even then, a variety of special techniques are required to sample, prepare, and label or stain the cells for meaningful observation and analysis. Cells can be counted in this manner, but often indirect methods of detection and quantification are preferred. DNA can be extracted from samples and several methods used to compare diversity by PCR amplification and/or enzymatic digestion (DNA “fingerprints”). For a more detailed description of current techniques for measuring prokaryotic diversity, I recommend the article by Vigdis Torsvik (3). Torsvik was one of the first microbial ecologists to apply molecular genetic techniques to the comparative study of microbial communities.

Guiding Students through the Steps of the Exercise

Step One

I recommend that you have students work in groups. Pass out transparencies, markers, and handouts first and do not pass out community diagrams. At this stage you may use the PowerPoint to project an image of one community so that students understand what to expect. In order to establish sampling “rules,” students will need your help in deciding how to establish plots.

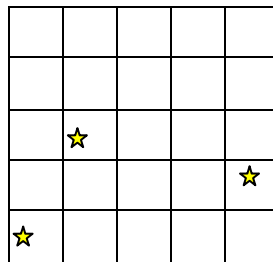
A simple approach for this activity will be to randomly place squares or frames onto the sampling area. In our case, students will overlay their black clear transparencies on the diagram of a sampling area. In the field, ecologists carry flexible wire, plastic, or wooden frames and toss them out into the sampling area in a randomized way.



It is a very good idea to discuss other more rigorous methods with students. For example, a very common and valid approach is to measure out the entire sampling area into blocks of equal size, number them as in a grid, then use a random number generator to select a subset

that can be reasonably sampled given time and resource availability. Note that plots of equal size like this are also referred to as quadrats, see definitions below.

In ecology a plot is a term used to describe a geographic space from which data are collected. Plots can be of varying size and shape, depending on the goals of the study. Ecologists typically collect data from plots of known sizes, referred to as quadrats—which can be any size or shape. If an area is too large for data to be collected throughout, a grid may be overlaid on the plot and data collected within randomly selected sections of the grid. Below is an example of a grid that may be overlaid on a sampling area. Each square can be numbered, then some are randomly selected as plots, in this case also quadrats, to be sampled. In the field, ecologists often walk out plot lines with a tape measure if this approach is utilized. Plots can also be defined by GPS coordinates. Imagine the following grid labeled with numbers from 1 to 25; five plots are randomly picked to be sampled. Random numbers can be drawn from an envelope of 25 pieces of paper or selected using a random number generator such as those found at <http://stattrek.com/Tables/Random.aspx> or other publically available Internet sites.



Step Two

The distinction of “taxa” will involve student judgments about color, shape, and size. It is worth bringing up the point that this may work for a simulated community on paper but is quite inadequate in defining prokaryotic taxa in nature. This is a good time to review how prokaryotic taxa (at the species level) are distinguished! There is a PowerPoint slide that addresses this, and I also call your attention to the article by Cohan (1).

Steps Three and Four

a. How is this community different from one that might exist in the water column of a lake, for example?

In the water column of a lake, organisms are not stably arranged. Water currents and transient temporal changes (e.g., diurnal sunlight) will change the location of individuals and clusters, sometimes randomly. Attached organisms are more permanently organized spatially and temporally. Changes will be reflected metabolically but not in community composition or structure.

b. If you were to imagine a habitat that this simulated diagram might represent, what would it be?

Some of the simulated communities are complex and dense; others are simpler and less dense. Soil communities would have a high degree of complexity (diversity) with perhaps clumped patterns in arrangement of cells. Bacteria on the surface of a catheter will be less diverse and perhaps more predictably arranged, maybe along a gradient of nutrient availability or temperature. By giving examples of this sort, you will help students brainstorm about communities they can imagine or may encounter and the diversity and structure they expect to be associated with such communities.

c. When sampling a community of this sort, does the scale of the area of investigation matter? In what ways will it affect your sampling approach?

Scale most certainly matters. Even though many microbes are particle attached and arrange themselves in predictable ways on the surface of the particles, this scale of organization is almost never of interest when studying larger-scale issues of ocean, lake, river, or soil diversity. For example, soil moisture, temperature, and organic content will drive large differences in microbial community composition and structure when different agricultural areas are compared. Small scale heterogeneity within each of these samples is irrelevant in that it does not contribute to the variation of interest.

d. If you would take data from nine small plots instead of the three relatively large plots you just did (adding up to the same comparable area), which sampling approach do you feel would give a more realistic or accurate picture of the actual community? What are the advantages and disadvantages of each approach? Do you think each method would give the same calculated Simpson's index? Why or why not?

Students will hopefully observe that by using several smaller plots, they are more likely to realistically assess community structure, especially in a heterogeneous environment. However, the diversity index may not differ significantly with plot size. If student groups do not all observe and draw these conclusions, you can use the PowerPoint presentation Part I to show the Sample Data set which does illustrate these points. In the final wrap-up with PowerPoint presentation Part II, you will see there is a very clear example from the literature (2) in which this sampling hypothesis is tested.

e. Finally, would a line transect approach be appropriate for sampling this community? Why or why not?

See the transect discussion below before initiating this topic with students. They will have some ideas at this point and will be in a better position to discuss transect sampling as compared to quadrats after they have actually done it.

Step Five

A standard method for transect sampling is called the “point method.” One determines a number of points, distributed randomly or regularly in the survey area. For example, these may be the intersecting points in the grid students established earlier. Randomly chosen coordinate pairs can be used to define transect lines. In discussion of transects, it should be pointed out that for microbes, samples are taken along the transect, but identification is not done in the field as would be done with plants. The same considerations that were made in plot-quadrat sampling should also be taken into account when determining line transect length, placement, and sample frequency along the transect.

Line transects are perhaps most useful when aligned along a known environmental gradient. *In lakes, depth sampling is basically vertical transect sampling and is highly appropriate based upon known layering of communities vertically in the water column. Other common transect scenarios include sampling along a gradient of altitude or with distance from a point source of contamination. In these cases, placement of the transect addresses the research question or hypothesis and is not random.*

Step Six

For each of your three sets of sampling data, calculate Simpson’s index of diversity. It will be helpful to use the Excel spreadsheet on your computer as a starting point. Do you obtain the same index using each of the sampling approaches? If there are differences, explain possible reasons why this occurred.

Wrap-Up and Class Discussion

Based on your findings, which sampling approach was more satisfactory in describing the actual community composition and structure? Note, Simpson’s index alone does not describe structure and organization.

At the end of this exercise, it may be useful to use the PowerPoint presentation Part II to help students review the concepts learned and point out some actual studies in the literature.

The PowerPoint slide illustrating the Fenchel and Findlay sampling experiment is informative when students are comparing all of the data sets. They tested different sampling approaches based on quadrat size and number (in this case cylindrical). It will be interesting to note whether or not students’ data aligns with their findings.

References.

1. **Cohan, F. M.** 2002. What are bacterial species? *Annu. Rev. Microbiol.* **56**:457–487.

2. **Findlay, S.** 1982. Influence of sampling scale on apparent distribution of meiofauna on a sandflat. *Estuaries* **5**(4):322–324.
3. **Torsvik, V., L. Ovreas, and T. F. Thingstad.** 2002. Prokaryotic diversity—magnitude, dynamics, and controlling factors. *Science* **296**:1064–1066.