

Using Microbial Ecology to Teach Experimental Design and Sampling Methods

Mary E. Allen and Ruth A. Gyure

Exercise 1: Student Handout Measuring Diversity

Finding a pure culture of microorganisms in a natural environment is virtually impossible, as most microorganisms inhabit spaces also occupied by other species. The assemblage of species that occur together in an environment is called a community. Communities are important ecological units because they are different in organization and function than their individual species components. For example, food webs, nutrient cycling, and succession are all characteristics of communities. These characteristics will vary depending on the particular assemblage of species present. Therefore two important aspects of community ecology are the study of species interactions (predation, competition, parasitism, etc.) and species diversity.

To determine diversity of a community, an ecologist must decide upon the factors that will be used to distinguish members of the community. Most commonly we think of species diversity, the number of different species of organisms represented. Communities can also be divided up based upon other characteristics of their component organisms. Photosynthetic microorganisms in an aquatic community, for example, can be grouped according to the particular kinds of photosynthetic pigments they utilize. Each group would likely contain more than one different species of organism, but the group would share a common set of characteristics that set it apart from other groups. This is an example of dividing a community into operational taxonomic units (OTUs). OTUs are defined by the investigator of the community and depend on the question being asked. They are especially useful in communities where species identities are difficult to determine.

To acquire a good measure of diversity, communities must be sampled often enough that all of the different species or OTUs are detected, including those that are rare. So how many samples are enough? This will vary depending on how many species or OTUs are present in a community and in what numbers. Ecologists use a species sample curve to measure whether they have adequately sampled an environment. An example of such a curve is shown below (Fig. 1). The graph depicts the number of new species detected each time an additional sample is collected. When no more new species are encountered, the ecologist can be reasonably satisfied they have detected most of the species diversity in a sample.

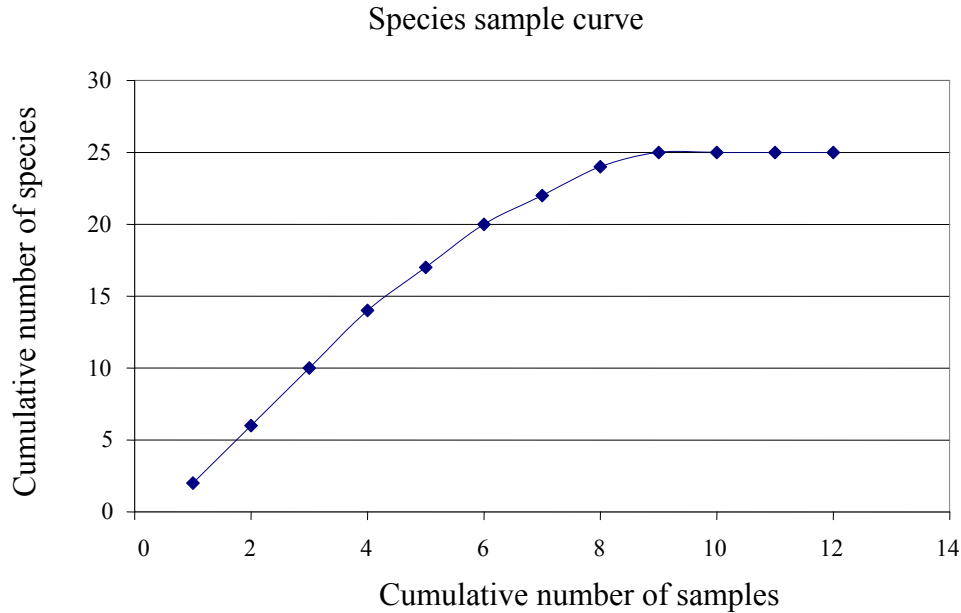


FIG. 1. This graph shows that the more one samples, the higher the likelihood of “finding” all species present. At some point, in this case after about eight samples are collected, additional samples will no longer reveal additional species—and the line levels off.

Many times ecologists want to compare the diversity of multiple communities. To do this accurately, a quantitative measure of diversity is necessary. Diversity is described by two quantitative measures: species richness and species evenness. These concepts also apply to OTUs, but for simplicity we will refer to them in terms of species diversity. Species richness (Fig. 2) expresses the number of different species that occur in a community.

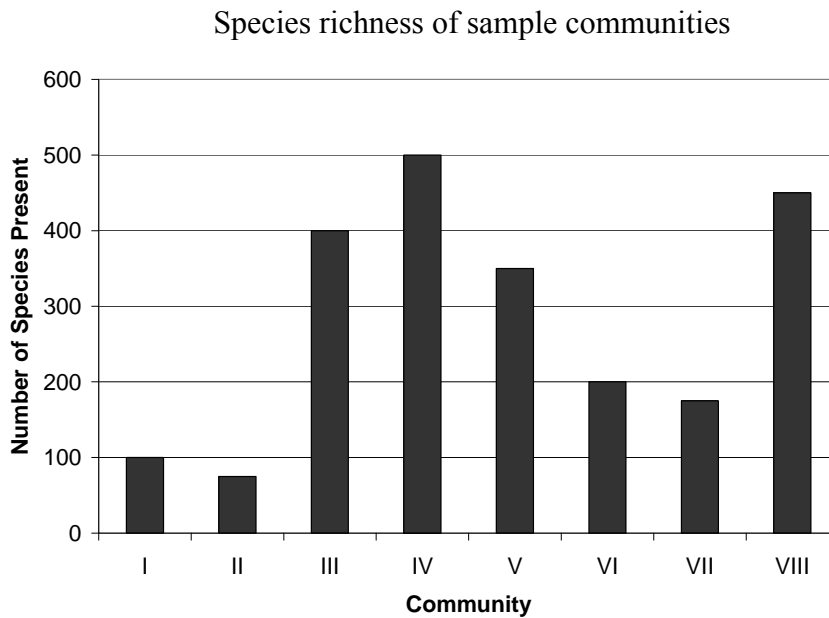


FIG. 2. The higher the species richness of a community, the greater its diversity. Community IV, for example, has the highest species richness, and by this measure alone would be considered most diverse of the eight communities represented.

Species evenness (Fig. 3) expresses the relative abundances of individual species within a community. For example, a community may contain 100 species (high species richness), but if one of these species comprises 90% of the individuals, that one species has a disproportionate influence on the functioning of the community.

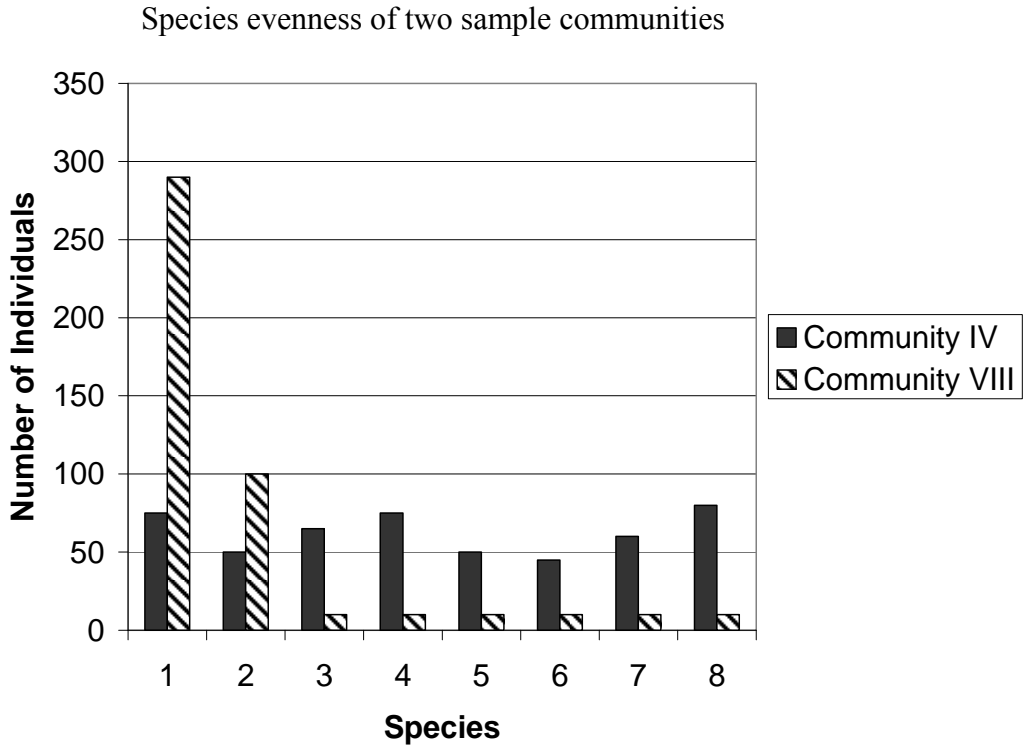


FIG. 3. Of the two communities represented here, Community IV has higher species evenness and therefore higher species diversity.

Measures of species diversity that consider both species richness and species evenness are useful for comparisons of different communities. Such values provide a measure of the complexity of a community. Communities with higher values may have more complex and varied sets of interactions among member species.

One method for quantifying species diversity is the Simpson’s index. Simpson’s index (D) is represented by the following equation:

$$D = 1 - (\sum (n_i/N)^2)$$

where n_i is the number of individuals of species I, and N is the total number of individuals of all species. The calculation of n_i/N is referred to as proportional abundance (p), because it is a measure of the proportional representation of each species in the community. These values are squared and then subtracted from 1, resulting in values that range from 0 to 1, with higher numbers representing higher diversity.

The following exercise demonstrates how this mathematical formula can provide us with a means for comparing the structure of different communities.

Sampling a community: how many samples are enough?

1. You have been given a “bag community.” Without opening the bag, record the letter identity of your community next to the heading for Table 1 on the Data Sheets.
2. Without looking into the bag, sample individuals, one at a time, from your bag community as many times as indicated by your instructor. As you draw out each individual from the community set it down beside you; do not return it to the bag.
3. Keep track of the number of times you sample your community and whether the type of individual (“species”) you sample each time is new using Table 1. Each time you collect a new species (one you have not yet encountered) from your community, indicate this by finding the row that corresponds to your sample number and enter the numeral 1 in the column labeled “Number of new species.” If you sample a species you have already drawn out of the bag previously, it is not a new species and you should write the numeral 0 in the “Number of new species” column. Keep track of the cumulative number of new species you sample from the community in the last column.
4. Once you have sampled your community the number of times instructed, construct a species sampling curve from the data you have collected using the sheet of graph paper provided to you. The x-axis represents the number of sampling events (e.g., 1 to 10), and the y-axis represents the cumulative number of species identified at each sampling event.
5. Look at your graph and determine whether you adequately sampled the diversity of your bag community. How can you tell if you did or did not?

Measuring diversity of a community: which one is more like the other?

1. Remove all members of the community from the bag and spread them out so you can see them. Group each “species” into its own separate pile. Determine the number of each species that you sampled and record these in the Simpson’s Index Data Table.
2. Determine the species richness of your community. Using the graph paper provided make a bar graph to demonstrate the species evenness of your community. Compare your measures of diversity to those of other groups in your class. Compared to others, did your bag community have high species richness? What about species evenness?
3. Now you can calculate a quantitative measure of diversity, which will allow you to compare the diversity in your community with those of other groups in the class. Remember that Simpson’s index (D) is represented by the following equation:

$$D = \sum (n_i/N)^2$$

where n_i is the number of individuals of species I, and N is the total number of individuals of all species. The calculation of n_i/N is referred to as proportional abundance (p) in the data table because it is a measure of the proportional representation of each species in the community. These values are squared and then subtracted from 1, resulting in values that range from 0 to 1, with higher numbers representing higher diversity. Determine proportional abundances and p^2 values for each of your species. Using the equation provided below the table, determine Simpson’s index of diversity (D) for your community. Compare this value to those your classmates have for their communities.

Data Sheet

TABLE 1. Species sample curve data for community

Sample number	Number of new species (0 or 1)	Cumulative number of species
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

TABLE 2. Simpson's index data table

Species	Total number	Proportional abundance (p)	p^2
Total			

Proportional abundance is the number of individuals of a species/total number of individuals

Simpson's index of diversity = $1 - (\text{total of the } p^2 \text{ values}) = \underline{\hspace{2cm}}$