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Aspects of Diversity Measurement for Microbial Communities

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A useful measure of diversity was calculated for microbial communities collected from lake water and sediment samples using the Shannon index (H') and rarefaction [$E(S)$]. Isolates were clustered by a numerical taxonomy approach in which limited (<20) tests were used so that the groups obtained represented a level of resolution other than species. The numerical value of diversity for each sample was affected by the number of tests used; however, the relative diversity compared among several sampling locations was the same whether 11 or 19 characters were examined. The number of isolates (i.e., sample size) strongly influenced the value of H' so that unequal sized samples could not be compared. Rarefaction accounts for differences in sample size inherently so that such comparisons are made simple. Due to the type of sampling carried out by microbiologists, H' is estimated and not determined and therefore requires a statement of error associated with it. Failure to report error provided potentially misleading results. Calculation of the variance of H' is not a simple matter and may be impossible when handling a large number of samples. With rarefaction, the variance of $E(S)$ is readily determined, facilitating the comparison of many samples.

In a recent report, Martin and Bianchi (16) supported the utility of diversity indexes as measures of microbial community structure. Such indexes have been approached only rarely by microbial ecologists (1, 7, 8, 10, 12, 14, 16), presumably because of the difficulties associated with the identification of bacteria. The methods of numerical taxonomy have been shown to function well in the construction of diversity indexes whether testing is extensive enough to allow grouping of isolates at the species level (7, 14), or a limited number of tests is used to generate clusters of bacterial strains related less intimately than at the species level (1, 16).

Limited testing has several distinct advantages. It allows for a greater number of isolates to be considered with no increase in the total amount of testing. Furthermore, as discussed by Mills (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N6, p. 139), the tests may be selected for their representation of important ecosystem processes, providing valuable information about the function of the community as well as about the structure.

In the microbiological studies which have employed diversity indexes for communities collected from the environment, the parameters used for the determinations appear to have been selected on an arbitrary basis. It is the purpose of the present paper to analyze the methodologies associated with construction of diversity indexes, particularly for nontaxonomic schemes; to provide an understanding of the limitations of such indexes; and to help subsequent investigators to generate meaningful results of diversity studies in a variety of habitats and environmental situations.

The procedures for measurement of diversity described here have been utilized as part of a study to determine the effect of dilute acid mine drainage on heterotrophic microbial populations in lake water receiving the waste. A portion of the data collected from that study is used to illustrate the properties of diversity indexes and is presented to demonstrate the utility of the nontaxonomic approach to calculation of such indexes.

MATERIALS AND METHODS

Sampling. As a part of a comprehensive study of the microbiology of the study area, enumerations of aerobic heterotrophic microorganisms in water and sediment at three stations (designated 1, 7, and 11) were made by the spread plate method using nutrient agar (Difco) as the plating medium. Counts were made each month; collections for diversity measurements were made on a quarterly basis. At each station, surface water samples were collected with sterile polypropylene bottles. Sediment samples were collected at each station with an Ekman dredge, placed in polyethylene bags, and returned to the laboratory for plating.

Collections of isolates for use in the diversity studies were made by selecting random colonies from the plates and preserving the microbes in culture on nu-

trient agar slants. In order to simulate the effect of different concentrations of organisms in equal volume samples from different stations, the number of colonies selected was not the same for all samples, but was in direct proportion to the number of colonies appearing on the plates. Thus, the sample size (number of isolates taken) was proportional to the size of the population at each station. Such a comparison may be made within water samples or sediment samples, but, since countable plates were of highly dissimilar dilutions, the relationship does not hold true for water samples as compared to sediment samples.

Characterization and clustering of the bacterial strains. Each station was examined for a total of 19 unit characters, most of which were selected for their relationship to important ecosystem processes (Table 1). All test media were inoculated using cells that had been grown in nutrient broth until the culture was slightly turbid, i.e., for 1 to 2 days at 25°C. Nutrient broth (Difco) or nutrient agar was used as the basal formulation for all of the test media. Unless otherwise specified, all incubations were carried out at 25°C for 3 days.

Growth at different pH values was determined by inoculating tubes of nutrient broth at the appropriate pH (4.0 or 7.0) and by incubating the tubes for 14 days. The additional time was necessary to allow development of many organisms that grew only slowly at the lower pH. Cultures grown on nutrient agar containing 10% soluble starch were flooded with an iodine solution to indicate hydrolytic activity. Lipolytic activity was determined by the formation of turbidity in a medium comprised of nutrient salts and corn oil as described by Mills et al. (17). Growth in and liquefaction of gelatin were determined by methods outlined in the *Difco Manual* (4). Resistance to the heavy metals (Zn, Cd, Cu, and Pb) was assayed by inoculating each of the strains onto nutrient agar amended with 1.5 mM Zn^{2+}, 0.09 mM Cd^{2+}, 1.5 mM Pb^{2+}, or 1.0 mM Cu^{2+}. Assays for NO_3^- reduction and denitrification were done by the method of Focht and Joseph (6). Cellulose degradation was determined by the method of Dubos (5). Utilization of acetate, citrate, glutamate, glycine, or serine as a sole carbon source was determined by observing growth of the isolates on a medium consisting of 0.2% (wt/vol) of the compound added to the mineral salts solution used by Mills et al. (17) and solidified with 1.5% purified agar.

TABLE 1. *Unit characters determined for each isolate used in assessment of microbial diversity*

Test no.[a]	Test
1	Growth at pH 7
2	Growth at pH 4
3	Reduction of nitrate
4	Denitrification
5	Lipolysis
6	Growth on solid medium containing starch
7	Starch hydrolysis
8	Growth in nutrient gelatin
9	Proteolysis
10	Decomposition of cellulose
11	Resistance to Cu
12	Resistance to Zn
13	Resistance to Cd
14	Resistance to Pb
15	Growth on acetate
16	Growth on glutamate
17	Growth on glycine
18	Growth on citrate
19	Growth on serine

[a] The test numbers are given for later reference where numbers instead of test names are used, e.g., Fig. 1.

For each strain, the characters were coded "1" for positive, or present, "0" for negative, or absent, or "9" for results which were uncomparable. Clustering of the isolates was accomplished by means of the program for numerical taxonomy, TAXAN 6, provided by Rita R. Colwell. The coefficient used was *S(SM)* (simple matching), and clusters were formed by means of unweighted average linkage.

Calculation of diversity indexes. Two methods of calculating diversity were used, the Shannon information index (21), and the rarefaction technique of Simberloff (22). The Shannon index (referred to as H') is defined by the equation: $H' = -\Sigma p_i \log p_i$, where p_i represents the number of individuals in a cluster (or species) divided by the total number of isolates in the sample being analyzed. Theoretical considerations offered by Pielou (19, 20) have demonstrated that H' is the best descriptor of population diversity. The index is the most commonly employed measure of diversity and has been used for several studies of microorganisms (1, 14, 16, 23). Except for comparisons with results of other workers, the base of the logarithm used for the calculation is irrelevant to the conclusions. Bases of 2, 10, and e have been used frequently, but the microbiologists have thus far used only base 2 (1, 14, 16). To maintain consistency, we used base 2 for the calculations described in the present paper.

The second means used for diversity calculations was the rarefaction method of Simberloff (22), a technique particularly suited to diversity evaluations of different-sized populations or samples. The results are expressed, not as an index as with H', but rather as the expected number of species (or groups) in a sample of an investigator-determined size. The same sample size may then be dictated for each collection, making comparisons simple. Simberloff (22) has provided a computer program for determining diversity using rarefaction when the investigator supplies the number of individuals in each different group. The basis of rarefaction is the construction of a curve for the sample which plots the cumulative number of phena encountered as increasing numbers of individuals are added from the community to the sample. From the curve, the expected number of species $[E(S)]$ or groups may be readily determined.

Properties of diversity estimators. Analyses were done to determine the effect on H' of the level of similarity used to group the isolates into clusters, and to determine the effect of the number of isolates used on that indicator of diversity. For the purpose of the present analysis, the data obtained from the sampling of October 1978 were used. In particular, 130 isolates from water samples of a single station (station 11) were used since the large number of isolates could be

subdivided and rearranged to facilitate the testing without introducing a bias of mixing in organisms originating from another sampling station or sampling time. Since all of the test results were recorded on computer cards, one for each isolate, the cards represented the microorganisms and could be shuffled, sampled, or reassembled quite easily.

To determine the effect of the number of tests used on the value of *H'* obtained from the resulting clusters, 50 isolates were randomly selected from the 130 strains collected from station 11. The isolates were grouped into clusters using 4, 6, 7, 8, 10, 12, 15, and 19 of the tests on the cards. In addition, the value of *H'* for all water samples taken in October 1978 was determined using 11 and 19 tests. For these analyses, the organisms were clustered at 100% similarity.

The effect of the number of isolates on diversity was determined only for *H'* since the rarefaction procedure is designed specifically to accommodate various sample sizes. From the deck of 130 cards representing station 11 organisms, samples of various sizes were drawn and clustered with TAXAN 6. Subsequent samples were taken with replacement of the cards removed in the previous sample.

The effect of the level of similarity on measured diversity was approached two ways. For *H'*, several of the analyses previously described were conducted at 100, 90, 85, 80, 75, 70, 65, and 60% similarity. Tables were then constructed which compared the parameter under examination at each of the similarity levels used. Rarefaction was used in a much different way. Since the computer program provided in the report by Simberloff (22) can be used to generate curves of expected species accumulation as more individuals are encountered, this technique was used to generate curves of cumulative cluster distribution obtained for samples clustered at several levels of similarity. A suitable percent similarity for subsequent analyses was chosen based on the shape of the resultant curves.

RESULTS

When the 130 isolates from station 11 water samples were divided into 5 equal-sized groups of 26 individuals each, the values of *H'* obtained were nearly identical for each group (Table 2). The level of similarity at which the isolates were clustered had little effect on the variation of those values, although *H'* decreased as the percentage of similarity was lowered. Since the subsamples were representative one of another for any given level of similarity, it was concluded that the random selection method was valid for obtaining smaller sizes from large groups of isolates. Unless otherwise stated, therefore, such subsequent simulations were based on a single random withdrawal of cards from the appropriate computer deck.

The number of tests used to characterize 50 isolates selected from the station 11 samples was of prime importance in determining the number of clusters and, consequently, the value of *H'* obtained (Fig. 1). Although the rate of increase for each value appears to lessen after about 14 tests were added, the apparent leveling off of the curve may not be real, but may rather be a reflection of the order in which the tests were added, in that not all tests have similar abilities to fragment clusters. For example, the 15th test added, growth on actate, produced in that position no new clusters and no increase in *H'*. While the numerical value of *H'* certainly was affected by the number of characters examined, Fig. 2 indicates that conclusions drawn from results obtained with the use of more or fewer tests were essentially the same; that is, diversity of aerobic heterotrophs derived from water samples and clustered at 100% similarity was highest at station 11, lowest at 7, and intermediate at station 1.

In order to establish an appropriate level of similarity for clustering the isolates, the computer program for rarefaction (22) was used to generate plots comparing the cumulative number of clusters encountered as the number of isolates used was increased (Fig. 3). At the 100% level of similarity, the accumulation of clusters was still increasing rapidly when 130 strains were

TABLE 2. *Value of H' obtained from equal-sized subsamples drawn from 130 isolates collected from station 11 in October 1978 and clustered at various levels of similarity*[a]

Subsample	Level of similarity (%)							
	100	90	85	80	75	70	65	60
A	4.35	3.58	2.59	1.83	1.33	1.33	0.40	0.00
B	4.35	3.75	2.89	1.99	1.36	0.86	0.70	0.70
C	4.45	3.75	3.12	2.39	1.83	1.03	0.40	0.40
D	4.29	3.85	3.19	2.26	1.93	1.20	0.40	0.50
E	4.22	3.39	2.72	2.46	1.40	1.30	0.93	0.93
Mean	4.32	3.65	2.89	2.19	1.57	1.14	0.60	0.50
± 1 SE[b]	0.033	0.10	0.10	0.12	0.13	0.09	0.10	0.17

[a] Subsamples were drawn without replacement.
[b] SE, Standard error.

included, indicating that not all of the clusters present had been sampled. At 70% similarity, 9 clusters were obtained, and 5 of the 9 were obtained after only 19 individuals had been included. With the testing scheme employed in this study, a level of 80 to 85% produced a curve which increased only slowly at 130 strains; the small rate of increase indicates that the level should not go much higher. At 75%, the curve became flat at around 30 strains, while at 80% the rate increased rapidly until about 40 strains were included. The leveling off of the curve indicates that the diversity value obtained will not appreciably increase, even with a much larger sample size. That is, by using the procedures described, essentially all of the groups have been sampled. Therefore, a level of 80% was used for subsequent analyses in the present study.

When *H'* was used as the measure of diversity, the size of the sample, i.e., the number of isolates,

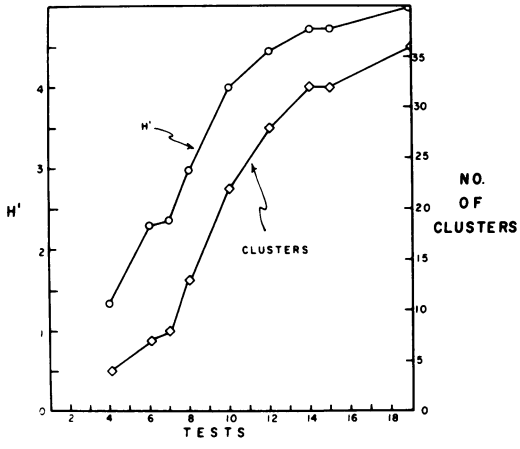

FIG. 1. *Dependence of H' and number of clusters obtained on the number of tests used for clustering. The numbers on the abscissa indicate not only the number of tests used but also identify the individual tests used according to the list presented in Table 1.*

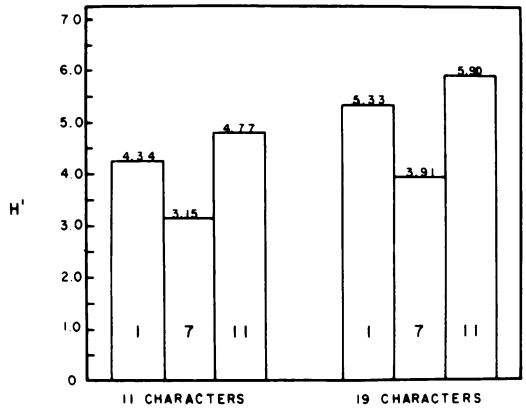

FIG. 2. *Effect of clustering with different numbers of tests on the pattern of H' obtained from communities of microbes collected from water samples from each of three stations. The numbers at the top of the bars are the actual H' values for the station; the number inside each bar is the station.*

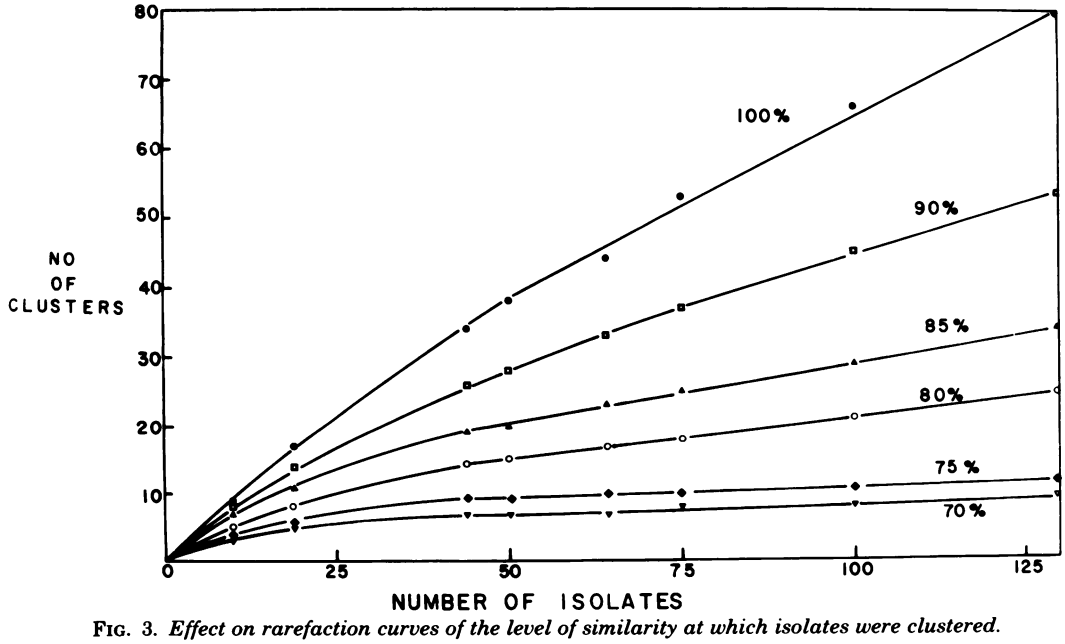

FIG. 3. *Effect on rarefaction curves of the level of similarity at which isolates were clustered.*

used to form the clusters exerted a strong influence on the values obtained. In a single sample, that of the October 1978 water samples from station 11, the value of H' varied directly as did the number of isolates used (Table 3). At some levels of similarity (100% and 90%) the relationship appeared to be linear. At other levels, the correlations were not so high that the relationship could be termed linear, but a strong positive correlation was observed at nearly all the levels examined. The strength of the correlation generally decreased as the level of similarity used was reduced; however, even at 65 to 75% similarity the correlation was quite high. A similar effect was observed when several different samples containing various numbers of isolates were compared for diversity. Samples with a larger number of isolates usually had a higher value of H' (Table 4). Correlations between the number of strains used and the value of H' obtained were not as high as those derived from subsamples of a single sample (Table 3), but the correlation coefficients showed that large samples tended to possess large values of H'.

A comparison of diversity values obtained for each of the samples by rarefaction, by H' using the original number of isolates collected from each station, and by H' using a randomly chosen collection of 19 strains from each station, is presented in Table 5. For the water samples, the pattern of diversity was the same no matter which method was used, i.e., diversity was highest at station 11, intermediate at station 1, and lowest at station 7. For the sediment samples, however, values of H' for the equalized sample series produced a different pattern than did those obtained with the original number of isolates. When 42 and 57 isolates were used for station 11 and 7, respectively, station 7 had the higher diversity. Using 19 strains for each station produced a reversal of the pattern; station 11 had the higher diversity. Rarefying the samples at 19 strains produced a pattern similar to that observed when the original collection of isolates was used to calculate H'. When the variance associated with the rarefaction results was considered, no significant difference (at the 95% level of confidence) was observed between the two communities. The apparent reversal of diversity pattern for H' with a smaller sample size

TABLE 3. *Values of H' obtained from different-sized subsamples drawn from 130 isolates collected from station 11 in October 1978 and clustered at various levels of similarity*[a]

Sample size (no. of isolates)	Level of similarity (%)						
	100	90	85	80	75	70	65
130	5.90	4.90	4.04	2.92	2.25	1.95	1.16
110	5.80	5.04	4.01	3.49	2.70	1.33	1.19
100	5.37	4.67	3.51	2.27	1.80	1.41	1.31
75	5.27	4.47	3.38	2.09	1.88	1.27	0.93
50	4.94	3.94	3.19	2.50	1.45	0.92	0.68
19	4.01	3.61	3.51	2.65	1.97	1.56	0.77
10	3.11	2.15	1.76	0.73	0.72	0.72	0.72
Correlation coefficient[b]	0.942	0.900	0.772	0.652	0.734	0.648	0.871

[a] Subsamples were drawn with replacement.

[b] Correlation coefficient of the least squares regression line of number of isolates against the obtained H' for each level of similarity.

TABLE 4. *Values of H' calculated for several samples having different sample sizes*[a]

Sample	No. of isolates	Level of similarity (%)							
		100	90	85	80	75	70	65	60
1 water	64	5.35	4.72	3.45	2.72	2.19	1.59	0.37	0.23
1 sediment	19	3.52	1.93	1.53	0.27	0.00	0.00	0.00	0.00
7 water	44	3.92	2.96	2.56	2.03	1.20	0.73	0.17	0.17
7 sediment	57	5.32	4.25	3.55	2.89	2.23	1.89	1.49	0.70
11 water	130	5.91	4.92	4.05	2.92	2.26	1.96	1.16	0.00
11 sediment	42	4.29	4.09	2.82	2.59	1.16	0.93	0.17	0.00
Correlation coefficient[b]		0.86	0.49	0.85	0.46	0.74	0.79	0.64	−0.01

[a] H' has been calculated for clusters obtained at various levels of similarity.

[b] Correlation coefficient of the least squares regression line of number of isolates against the obtained H' for each level of similarity.

TABLE 5. *Diversity of heterotrophic microbial communities as measured by rarefaction and H' using the original sample size and samples adjusted to equal sizes*[a]

Sample	Heterotrophic count[b]	*N* (no. of isolates in original sample)	*NS* (No. of clusters in original samples)	*E*(*S*) (expected clusters with 19 isolates)	*H'* (*N* isolates)	*H'* (19 isolates)
Water						
1	47	64	15	7.96 ± 0.58[c]	2.72	2.15
11	752	130	24	8.03 ± 1.69	2.92	2.65
7	24	44	6	4.92 ± 0.76	2.02	0.63
Sediment						
1	80,000	19	2	2.00 ± 0.0	0.27	0.27
11	250,000	42	6	5.33 ± 0.58	2.58	2.55
7	350,000	57	12	7.62 ± 1.2	2.88	2.22

[a] Clusters were formed at the 80% level of similarity.
[b] Colony-forming units ml^{-1}.
[c] One standard error of the mean.

could be due to the fact that only a single subsample was withdrawn for each collection; yet, since the communities are not statistically different, the values of *H'* obtained, although misleading, cannot be considered as incorrect or inappropriate. On the basis of 95% confidence limits calculated from the variance associated with the rarefaction results, the stations exhibited no significant differences in diversity, with the exception of sediment from station 1, which was significantly different from all other stations examined. Unfortunately, no variances could be calculated for *H'* since an insufficient number of replicate samples were collected.

The trend in diversity of the microbial communities appeared to be related to the original community size; that is, large communities possessed a higher level of diversity, even though statistical significance could not be demonstrated. The observation was valid, however, only when similar habitats were examined; viz., water or sediment. Comparisons of water samples with sediment samples indicated that no difference in diversity exists between the two environments, in spite of the large difference in the numbers of organisms recoverable by plating on nutrient agar.

DISCUSSION

Equations used for the calculation of diversity are not constrained by the type of groupings used for strains. Depending on the sampling scheme, either of the described methods for calculating diversity, viz., *H'* or rarefaction, may be used to measure diversity after a dendrogram has been constructed. *H'* may be employed to compare communities or to provide a descriptive element for a single community; however, rarefaction is only useful for comparisons of two or more communities.

When using a diversity index based on information theory, the type of collection should dictate the appropriate index to be used (18). If, and only if, all of the individuals in a community are counted and classified, the diversity, Brillouin's *H* (2), is determined and not estimated, and there is no error associated with the value. For collections where all of the individuals are not identified, or where the community sample is considered infinitely large, so that an infinite number of samples may be taken without depleting the community, *H'* is the proper index (19). Unless all of the types of individuals are known for the community being sampled, it must be assumed that *H'* will be a biased estimator of the community's diversity, and the error of the estimate must be found (11, 13, 15, 18, 20). Although this situation applies to nearly all studies involving microorganisms, none of the microbiological diversity studies has reported errors associated with the calculated estimate of *H'*.

The variance of *H'* is computed on the basis of several samplings of the same community (11, 13, 15, 18, 20). Such a procedure is, at least, laborious and potentially expensive to carry out, particularly when *H'* is being calculated for several communities. The calculation of the variance of *H'* may be appropriate for comparisons of 1 or 2 sites, such as are frequently reported on by numerical taxonomists; however, for investigators dealing with many comparisons, it may be more convenient to measure diversity (and the error of the estimate) by rarefaction.

The use of levels of resolution other than species is not a novel concept to microbiologists examining diversity. Staley (23) has used similarities in colony morphology as a basis for grouping isolates into clusters for use with the Shannon equation. However, in terms of ecosystem function, it is the activities of the organisms that are of real interest, and a numerical taxo-

nomic approach, even without species level clustering, will frequently provide a great deal of information about the functions as well as the structure of a community. The use of a limited number of tests can provide appropriate clusters for diversity analysis as well as furnish information concerning the potential for the organisms sampled to carry out ecosystem functions of importance, interest, or both. Since the tests should be selected on the basis of their direct relation to important processes in the environment under investigation, little is to be gained by adding tests simply to differentiate isolates. In their study of algae-bacterial interactions, Bianchi and Martin (1, 16) used 53 tests to group the bacterial isolates into "profiles." While the number of characters examined was unnecessary for diversity analysis, the additional tests were used to formulate additional indexes related to the number of extracellular reactions carried out (exoenzyme average index) and the number of organic substrates used by the groups of microbes (utilization average index). Such an approach appears quite promising in terms of evaluating the ultimate ecosystem effect of stress on microorganisms and represents a distinct advantage that may be derived from the numerical approach to diversity analysis.

The effect of a reduction in the number of tests is, as has been demonstrated, a reduction in the calculated diversity, but not a change in comparative patterns of diversity. Further evidence that a limited number of tests provides adequate clustering ability is given by Debette et al. (3), who found that in classifying soil bacteria, the information obtained was not significantly affected by a reduction in the number of tests used from 51 to 21, and by Griffiths and Lovitt (9), who successfully used 9 tests to form profiles for examining bacterial diversity.

Perhaps the most significant effect on the calculated diversity value using H' was related to the number of isolates used to form the clusters. Although H' takes both species richness and evenness (the distribution of individuals among the species) into account, richness has a far more powerful influence on the value of H' than does evenness. Many of the samples contained a high proportion of single-member clusters, and the number of clusters observed tended to increase rapidly as the number of isolates used was increased. While the effect was most significant at high percent similarities, only slight relief was obtained by clustering at a lower percentage. If an investigator desires to use H' as the measurement of diversity, comparisons should be made on samples of similar size. For example, 50 random isolates from each sample could be chosen for comparison. Often, however, isolates lose viability during the testing procedures so that what began as equal-sized samples are not so at the end of the testing. In such cases it may be advisable to randomly withdraw isolates from the larger samples until all samples are the same size. While such a practice may introduce some error into the diversity estimates, the error will most likely be much less than that obtained if different-sized samples are used. A case of erroneous conclusions from a reduction in the sample size was evident in the change in the pattern of H' obtained for the sediment samples when the number of isolates was reduced from the original to create samples of an equal size (Table 5). Based on the use of H' alone in the equalized sample, one would determine that the microbial sediment community from station 7 was more diverse than that from station 11; however, the confidence intervals associated with the results of the rarefaction analysis indicate that the two communities have diversities that are not significantly different. Thus, the results of the two H' analyses are both correct, but misleading. The lack of a readily calculable variance of H' may be a severe drawback in the application of the index to microbial samples, at least for the sample size ranges that were used here.

For unequal sized samples, the rarefaction procedure may be applied to the data to provide meaningful results. In fact, since the distribution of individuals among the clusters is used to determine the expected number of species, rarefaction accounts for evenness as well as richness as pointed out by Tipper (24). Thus, if a suitable number of isolates cannot be obtained, or if some of the organisms are lost after isolation, differences in sample size may be rapidly and confidently corrected for.

An important criterion for any approach to diversity is the ability for individual investigators to compare results of their work. Obviously, it is critical for workers to have a complete understanding of the methods involved, since results obtained with the various techniques are not always comparable. Values of H' calculated using samples of widely different size may not be comparable. Furthermore, the base of the logarithm used to determine H' may be e, 2, or 10, and researchers must always indicate which base has been used for their calculations.

While the above considerations apply to both full taxonomic (species diversity) and limited taxonomic approaches to diversity, certain points are problematic only for the limited taxonomy procedures. Since a low number of tests (e.g., less than 20) will probably be used, it is important that direct comparisons be made only among groups clustered using the same tests and

the same number of tests. Some tests possess a greater ability to fragment clusters so that identical arrays of tests are required for direct comparison of the resultant indexes (Fig. 1). For descriptive or nonquantitative comparisons, any combination of tests is appropriate, and since it is assumed that a great many nonquantitative comparisons will be made, the test identities need not be limited to a standard set.

In order to properly compare results of various studies, certain information is highly essential; that information is the number of clusters and numbers of individuals within each cluster, whether the clusters be species or other phena. Staley (23) refers to the information as raw data and provides a format for its inclusion in a research article. Likewise, Table 6 presents the raw data (at the 80% level of similarity) used for the calculations made in the present paper. For those who publish results of numerical taxonomy studies, inclusion of the dendrogram will provide the raw data in an identifiable form.

The purpose of this paper is not to provide a standard method to be used universally in all investigations of microbial diversity. The hope is that many microbiologists engaged in ecological research will begin to investigate the use of diversity, through a variety of approaches, to describe microbial communities, their natural properties, how they change under conditions of natural stress, and how they respond to human alteration of the environment. Diversity, measured by any procedure, will never become a total answer to the determination of low-level stress effects, but when coupled with methods such as activity measurements, biomass evaluations, and other descriptors of community structure, the property may become a valuable addition to the arsenal of ecological tools held by the microbial ecologist and may be used to further understand the world.

TABLE 6. *Clusters (80% similarity) of organisms collected in October 1978*

Station	Sample	Raw data[a]
1	Water	1 (32), 3 (5), 1 (4), 1 (3), 1 (2), 8 (1)
1	Sediment	1 (18), 1 (1)
7	Water	1 (17), 1 (15), 1 (6), 1 (3), 1 (2), 1 (1)
7	Sediment	1 (16), 1 (12), 1 (10), 1 (5), 1 (4), 1 (3)
11	Water	1 (66), 1 (15), 1 (12), 1 (10), 2 (4), 1 (3), 3 (2), 2 (1)
11	Sediment	1 (19), 2 (6), 2 (5), 1 (1)

[a] Numbers in parentheses represent the number of individuals in a cluster; that is, the size of the cluster. Numbers outside the parentheses represents the number of clusters of that size.

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LITERATURE CITED

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