

NUMERICAL ECOLOGY: DEVELOPMENTS FOR MICROBIAL ECOLOGY

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INTRODUCTION

The working group first recognized that in microbiology we have two different but complementary topics where numerical methods are relevant:

- 1) statistical definition of taxonomic and/or functional entities,
 - 2) statistical descriptors and/or mathematical analysis of relationships between bacterial and environmental variables.
- Hence, we meet the problems of pelagic systems (c.f. Flos et al., this volume), benthic communities (Field et al., this volume) and those of general interest from limnology and oceanography (Legendre et al., this volume) as well as from terrestrial environments.

Our data matrices from taxonomic studies are generally in the form of matrices containing binary informations about various qualitative results from biochemical tests. The analysis of such matrices can involve first a taxonomic study, to replace a vector of binary biochemical results by a species name or number, followed by an analysis of the species-by-sites data table as in classical numerical ecology; or microbiologists may wish to analyze directly the isolates x biochemical descriptors x sites

data table, insofar as methods are available to do so. This is the peculiarity of microbiological data.

Environmental data include binary or ordinal variables both quantitative and qualitative describing an ecosystem, such as physical-chemical descriptors, numbers, ratios or allometric values (e.g. Schwinghamer *et al.* 1986).

Following is a list of the main problems specific to microbiological data.

1) Investigations on micro-organisms often require indirect methods, such as the estimation of uptake parameters of organic substances or the measurement of ATP as an indicator of overall active microbial biomass. Those methods, however, produce high variability and raise the question of the validity of these indirect methods. This holds especially true for the estimation of an "actively metabolizing" population when specific substrates are regarded as models for the description of general metabolic processes (Bölter 1982).

2) Further discussions in this working group considered that micro-organisms act at special scales in time and space with regard to their small size and metabolism, implying the definition of an adequate sampling scale (Troussellier *et al.* 1986a). This has been stressed by more holistic approaches to ecosystem analysis when micro-organisms were shown to be notable exceptions in the allometric relationships between particle size and their turnover rate (Azam *et al.* 1983, Field *et al.* 1985). As illustrated by Steele (1978), scales in ecosystems - both time and space - are indicative of the relationships between physical and biological processes (Legendre and Demers 1984).

3) Autocorrelation is one of the most important facts that have to be mentioned during sampling in the pelagic environment (c.f. Legendre *et al.*, this volume). Furthermore, the microbial environment is strongly patterned and during its analysis we have to consider various niches and size classes. Although physical descriptors may act over wide gradients and/or distances, the

distribution of organic material, which is the main controlling factor of microbial activity, is very patchy. This holds true for the pelagic environment as well as for the benthos or soils. Thus, it is difficult to fulfill statistical requirements during sampling strategies.

4) Another crucial point to be considered is the "translation" of ecological descriptors into numerical variables. This includes the above-mentioned high variability of ecological data. In some cases, variability may be expressed in statistical terms, such as standard deviation or variance. If so, these terms may be used in weighing individual parameters. Further methods include data transformations, like for instance conversion to ranks or to octave scale (Gauch 1977). This is of special interest with regard to high internal variability, to eliminate noise of original field data or to detect thresholds. This may have fruitful consequences in further analyses.

Most of the methods discussed below are well suited to describe ecosystems in different ways. However, we recommend that microbial ecologists try them on various data sets, and publish the results comparing the new methods with the more classical ones, in order to assess their relevance and applicability in the microbial world. The following will reflect the discussions of the working group with special reference to the topics of the main lectures given during the individual sessions.

MULTIDIMENSIONAL SCALING

During the discussions on the use of the techniques for multidimensional scaling (Gower, this volume), we recognized that these methods are applicable both to problems of fabricating species-like entities by means of numerical taxonomy, and to studies of the structure of microbial communities in relation to environmental parameters. When analyzing communities of different geographical or temporal origin the problem of overlap between populations arises leading to autocorrelation problems. This may

be due to a recurrent pattern in the communities or to a homogeneous "background" population.

In such case we can adopt the Procrustes analyses. This method also allows us to compare different measures which have been used to set a similarity matrix. Thus, we can elucidate various interrelationships between original variables or detect effects of noise. The comparison of results from multiple correspondence analysis with those from Procrustes analysis is of further interest when analyzing multivariate data sets.

R-mode Principal Component Analysis can be used to replace sets of correlated variables by a few synthetic but independent variables. It was mentioned that another approach to discover redundancy in numerical taxonomy is the establishment of "median strains" (Sneath and Sokal 1973) which may serve as a carrier of information of a group of bacteria. This has been applied during studies of marine and limnic bacteria by Böltter (1977) and by Böltter et al. (1986).

Q-mode analysis can be used on data matrices to obtain information on the metabolically active component of the population. This provides a functional description of a community. Problems of numerical taxonomy can be avoided by analyzing the multi-sample (isolates-by-variables) data table using canonical variables, or more simply by canonical coordinate analysis (Digby and Gower 1981) as described by Gower (this volume, section 7.4).

The partitioning of the original data matrix (e.g. into results from biochemical properties, or into results from antibiotics and sera) is recommended in order to compare the different results of grouping procedures. Such comparison can be carried out either by canonical correlations or by the Procrustes approach. It should, however, be kept in mind that any comparison by this method takes place at the level of the computed distances (or similarities) and not among the original data. A systematic comparison of results of these approaches should be done for the same data sets.

SCALING OF MULTIWAY DATA

Multidimensional scaling of multiway data (Carrol, this volume) can be used to solve problems of asymmetric ecological matrices, for instance by doubling the entries of the affinity matrices. This matrix may contain data on relationships between bacterial communities and their substrates or other environmental descriptors. A further advantage of this kind of scaling is that it can be computed from incomplete distance or affinity matrices, with the computer programs presently available.

The input data into a three-way matrix may include information about stations, times and environmental variables or data on the abundance of taxa. Such a data structure often occurs when analyzing survey data with different dimensions of i, j, and k. The data can be ordinal variables as well as ranked or binary data.

The results from such analyses will give ordinations of sites, times or variables, including taxa, and may lead to informations about successions or environmental gradients (Legendre *et al.* 1985a,b, Sprules 1980). However, as far as we know such analyses on three-way matrices have yet to be done. Special problems of interpretation may arise during the ordination of time-samples when the time span of the analysis runs over more than one biological cycle of the population (Legendre *et al.* 1985a,b). It should also be kept in mind that physical and different cycles of different scales of magnitude may act on micro-organisms.

The imprecision of quantitative measurements may have strong effects on the results of such analyses. For instance, the definition of microbial taxa by numerical taxonomy may have a high degree of uncertainty. This holds also true for many environmental variables and data on abundance. Scaling techniques can take the standard deviation of a measurement, or some other measure, as weighting factor.

The duality diagram (Escoufier, this volume) emphasizes the fact that ordination techniques offer the choice of weighting factors and data, and choosing the distance coefficients best suited for the goal. There is no statistical method to weigh variables a priori; weights can be obtained for instance from a panel of experts, by the Delphi method.

NONLINEAR MULTIVARIATE ANALYSIS

Data from microbial ecology are often in the form of continuous physical-chemical measurements, but may be species abundances or presence/absence data of functional groups. Since any transformation of a data matrix modifies its original information, the model adopted must be consistent with the original data in order to get a satisfactory structure. The representation space Y is a transformation of the data space X and is characterized by a loss of information (de Leeuw, this volume). This holds true for the analysis of both taxonomic data and environmental descriptors. By using the GIFI system of nonlinear methods, it becomes possible to analyze data which include mixed types of variables, such as nominal and ordinal ones as well as physical measurements.

Unfolding analysis (Heiser, this volume) may be useful in ecological studies under the assumption that (taxonomic) abundance data are replaced by data on microbial activity. Examples for possible applications of unfolding analysis may be found in the sedimentary environment where typical zonations of different types of metabolic activities occur (c.f. Rheinheimer 1981). Unfolding analysis of those data could offer an innovative approach to the description of interrelationships between micro-organisms and their environment. It would be interesting to compare this method with direct gradient analysis (Gauch 1982) or to see whether the relation between descriptors (e.g., aerobic and anaerobic activity), which exhibits a horseshoe effect in PCA, could be resolved into two unimodal distributions by unfolding analysis. In such an analysis, it is useful to emphasize the

guild concept rather than to describe taxa or even species. Thus the measure of abundance can be replaced by data on different types of physiological activities, in order to relate them to other environmental descriptors such as time, depth, etc.

Another use may be the analysis of stratified signals in sediments or at other boundary layers. The ordination of vertical or horizontal gradients in depth assemblages of, for example, foraminifera or diatoms may be improved by application of unfolding analysis, again in comparison to gradient analysis as applied by Cisne and Robe (1978). Furthermore, this technique may also be applied to the ordination of organic or inorganic residual compounds indicative of past microbial activities.

However, there may be limitations in the ecological interpretation of an unfolding plot. Unfortunately, we have no example of such analyses, or the study of the stability of the results in microbial ecology. A restriction of this kind of analysis may also be the existence of autocorrelation among descriptors of biological gradients or along time series. A correct specification of dimensionality of the model is crucial to the success of unfolding analysis. Stress diagrams (Carroll, this volume) are proposed to assess this dimensionality.

CLUSTERING OF FUZZY SETS

Ecological data handled by numerical methods often include probabilistic terms, as mentioned previously. Both environmental and taxonomic data include various sources of imprecision with unknown distributions. Especially data used in numerical taxonomy may be in the form of a "more or less" positive or negative response to a biochemical or physiological test. For instance, young cultures of bacterial strains may give results that are quite different from cultures of older strains, or characters may depend on culture conditions such as nutrient concentration or temperature. Thus, the final information in the data matrix may be to some extent uncertain.

Hierarchical clustering imposes clear distinctions among clusters while fuzzy clustering admits the uncertainty of the cluster space (Bezdek, this volume). Another use of fuzzy sets may be the analysis of ecological niches by environmental variables. This technique has not been applied in ecological studies at the microbial level, although the concept of fuzzy clusters applies very well to the ecological problem of species associations.

CONDITIONAL CLUSTERING

Conditional clustering (Lefkovich, this volume) with or without pairwise resemblances may be used well in the analysis of ecosystems described by occurrences of functional groups of micro-organisms, instead of using taxonomic units. This approach may be of special interest in identifying spatially separated communities comprising similar functional groups. Furthermore, it stresses the identification of similar complex structures rather than their partitioning into numerous subgroups. This is a particular advantage when analyzing tables (species-by-sites) in order to find the most probable associations by eliminating redundancy. In contrast to hierarchical clustering, conditional clustering looks for "true groups" in the original matrix. For the purpose of numerical taxonomy, conditional clustering has been used to choose relevant taxonomic features for the identification of yeasts (Lefkovich, pers. comm.). The algorithm is apparently efficient, which is important for its application to large data sets.

CONSTRAINED CLUSTERING

The application of constrained clustering (Legendre, this volume) is useful for the analysis of time series, spatially distributed data and environmental gradients when autocorrelation occurs. Users of such methods should make sure, however, that the sampling frequency exceeds the frequency of possible stochastic disturbance factors and environmental cycles.

The use of environmental constraints such as edaphic or nutritional spaces is especially promising. In some microbiological studies, time and geographic space may be considered jointly, for example during sampling at different instances along a river system. Likewise, different environmental gradients, such as vertical distributions of organic matter in the sediment or temperature/salinity gradients in a pelagic system may also be considered.

Another application of this method is the investigation of microbial processes at different time scales. In sewage plants, for example, time scales of various active microbial populations, i.e. their metabolic processes, are superimposed by externally determined time scales, e.g., the input of large amounts of sewage at certain times. An example of such a study is given by Legendre *et al.* (1985a). It has been mentioned that constrained clustering is rather stable compared to other methods in the sense that small variations in data, or changes in the clustering algorithm, are unlikely to produce large changes in the clustering results. It would be very promising to compare results from this approach with those from unfolding analysis.

There is an urgent need for comparison of results from different cluster algorithms used in ecological studies. Such comparison is crucial for the interpretation of any classification. In many cases, during such data analyses, the data are regarded as "hard" and they are analyzed under this assumption. However, one must define criteria with special reference to the analytical procedure.

FRACTAL THEORY

Fractal theory (Frontier, this volume) can be used to describe ecosystems in terms of their hierarchical structure. The working group discussed many examples in which fractal theory might be useful in describing features of ecosystems that are of direct interest in microbial ecology. For example, we know that

microbial activity is generally enhanced at boundary layers (e.g., Liebezeit et al. 1980) or in frontal systems (Lochte 1985), both of which can be described by their fractal nature.

Changes in the fractal dimension of a phenomenon may point to changing interactions which have to be considered in ecosystem analysis. This may be used to find the correct scales for measuring microbial activity. These scales may be defined by changes in the slope of the Mandelbrot plot describing spatial distribution of particles, e.g., in the benthic environment. In general, fractal analysis may aid in the ecological interpretation of any size distribution and related biological processes. Again, this method has apparently not yet been used in microbial ecology.

PATH ANALYSIS

Multiple regression models have been widely used in ecological studies (e.g., Dale 1974, Bölter et al. 1977). Classical path analysis as introduced by de Leeuw (this volume) uses linear regression type models to assess the validity of various causes-and-effect relationships. Schwinghamer (1983) used this approach in describing relationships in benthic microbiology, while Troussellier et al. (1986b) used it to model the behaviour of bacteria in an eutrophic ecosystem. The advantage of this method is the restriction to a limited model. This offers a method of setting hypotheses that can be further tested by field observations.

A valuable extension of this method is the incorporation of latent variables into the model. These are composites of observed variables which describe theoretical constraints that are not measured directly. Many processes in natural systems are linked by feed-back mechanisms. Path analysis models are not very well suited to model those systems in which such processes are dominant features. However, they can be introduced into the classical model using explanatory variables with a lag of (t-1)

for instance. Furthermore, classical path analysis is not adapted to handling non-quantitative variables, while non-linear path analysis can easily do so (de Leeuw, this volume).

SPATIAL AUTOCORRELATION

Analysis of spatial autocorrelation (Sokal, this volume) must be considered very carefully with regard to the scales of observation in microbial ecology. This is a major problem for sampling design common to most studies in microbial ecology, because the interactions of interest occur over a broad range of size and distance scales. Sampling strategies commonly used in microbiology (e.g., Colwell and Morita 1974) are not likely to avoid natural spatial autocorrelation completely. Spatial autocorrelation of microbes is important both in the range of a few micrometers (contagious growth), and at much larger scales.

Random sampling schemes may ensure the absence of sampling autocorrelation, yet the values of the variables may still be autocorrelated in space due to underlying processes. Autocorrelation must be tested for by methods such as outlined by Sokal (this volume), and disproved before usual ANOVA or correlation analyses can be done.

Plots of the Mantel statistics (Sokal, this volume) on distance classes or correlograms may be used to describe the autocorrelation structure, but care must be taken because correlations between distance matrices are not easy to interpret. The data surface may be reconstructed by contour maps. Trend surface analysis using polynomial regression, or kriging, may be used to analyze spatial patterns in autocorrelated data. An example in analyzing plankton community structure has been presented recently by Mackas (1984).

POINT PATTERN ANALYSIS

Point pattern analysis (Ripley, this volume) is not originally designed for microbiological purposes; however, it may be useful in many problems in microbial ecology, despite the fact that it is difficult to make direct observations of microbial populations and their distributions in the natural (undisturbed) environment. In fact, methods such as scanning electron microscopy have been used to get insight into this ecosystem (e.g., Zimmermann 1977). Other methods are mainly in use to find colonies of bacteria in the natural environment rather than describing their original distributional pattern. Those patterns on plates or filters (e.g., epifluorescence microscopy) are rather considered to be artifactual distributions. Nevertheless, they can contain information about interactions between growing colonies.

A possible use for this method has been recognized in the description of the pattern of physiological (functional) groups in the natural environment. In this case, abundances of functional groups or other variables describing microbial activities may be regarded as points distributed over a certain area. However, it is not known whether this approach in describing microbial communities has been carried out successfully. A special problem may arise with regard to unstable environments like the pelagic system in the oceans.

Ripley (pers. comm.) has mentioned the application of this method to the epidemiology and geography of human disease. Plant and animal diseases as well as other associations with microbes (e.g., in the rhizosphere) may also be studied by similar methods.

"Marked Point" methods are available (e.g., Diggle 1983) which allow point patterns to be related to other variables, discrete or continuous. Thus, distributional patterns of microbial functional or taxonomic groups may be analyzed in relation to other spatially varying factors. Given precise positioning techniques these methods may be useful in systems

with few stable structures, as mentioned for the marine pelagic zone. As such, point pattern analysis may be a more sensitive approach to detecting spatial patterns of microbial communities in nature than other currently available techniques which rely on spectral analysis and related methods. In addition, simulation methods are available to test the significance of cross correlations between spatial patterns of more than one variable.

CONCLUDING REMARKS

Only a few of the methods discussed above have yet found applications in microbial ecology. Though numerical taxonomy has been widely used in general microbiology (Baleux and Troussellier 1985), since its introduction to this field by Sneath (1957), even this method is rarely applied for ecological purposes. Oliver and Colwell (1974), Troussellier and Legendre (1981) and Legendre et al. (1984) tried this method in describing fluctuations of microbial populations. Böltter (1977), Witzel et al. (1980) and Böltter et al. (1986) used this method for taxonomic purposes in the marine and limnetic environments.

Hierarchical cluster analysis has also been used in structuring correlation matrices from variables of microbial ecology (Böltter et al. 1981, Böltter and Meyer 1983). Only a few attempts are known, however, in using non-hierarchical clustering on microbiological data (Böltter and Meyer 1986) or constrained chronological clustering (Legendre et al. 1985b).

This lack of applications of numerical methods in microbial ecology holds also true for other methods. Schwinghamer (1983) introduced path analysis, while Troussellier et al. (1986b) used this approach in analysing biological wastewater treatments.

Many other methods discussed during the sessions seemed to be very promising for use in microbial ecology. However, the working group could not go further than making recommendations to ecologists for adopting methods like scaling techniques. For many

of the other methods like unfolding analysis, fractal theory or point pattern analysis, their value for ecological purposes will be known only after they will have been applied to many real problems.

The working group thought that it was a great advantage to obtain knowledge about these advanced mathematical methods and to introduce them to ecological science. We would like to encourage more microbiologists to get in closer contact with people who are familiar with these methods. This would yield new insights into the system of the micro-organisms due to the stimulation from methods that help generating hypotheses which complement the more usual method of hypothesis testing.

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